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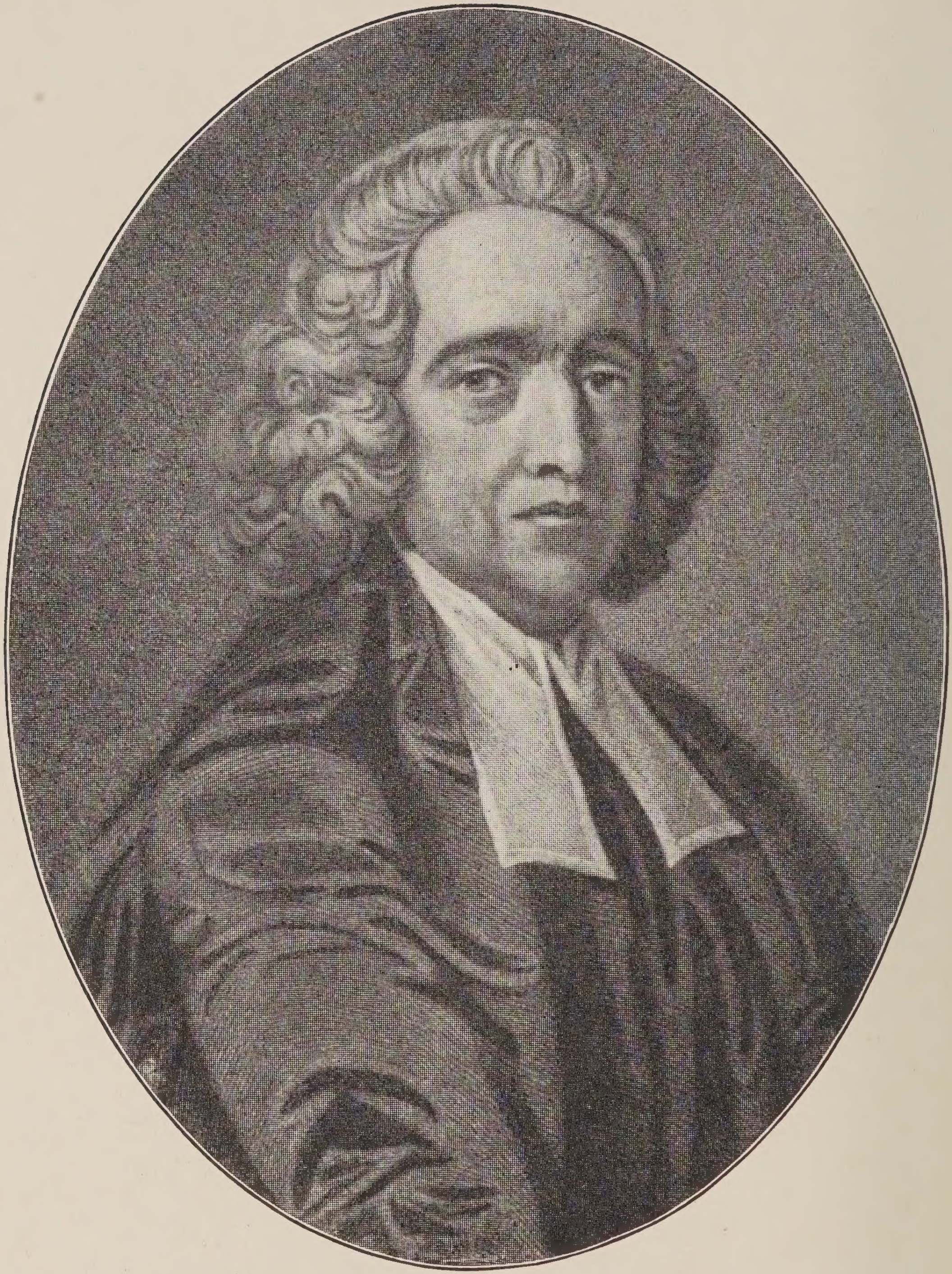
THE PHYSIOLOGY OF PLANTS



Stephen Hales, 1677-1761. (From J. Cederquist.)

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THE
PHYSIOLOGY OF PLANTS

BY
Ernst

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PREFACE

Classical textbooks on the physiology of plants, such as the monumental works of Pfeffer and Jost, served botanists long and well as reference volumes, but there soon arose the need of shorter treatises for purposes of instruction. One of the first books to fill this requirement was written by S. H. Vines in England. Similar textbooks have followed it. Excellent as these are, there still remains, if not a need, then certainly room for a book handling the subject in a somewhat different way.

The present volume has been written for the college student who has a background in general botany, physics, and chemistry. It is written with the hope that the student will gain an acquaintance not only with experimental data, but also with an understanding of the principles and problems in plant physiology. An encyclopedic assemblage of facts is for the advanced worker. This does not mean that experimental facts are not for the beginning student; on the contrary, they are the basis of all science and so come first; but too often the student is left with facts only, and given no encouragement to think about them. Theory supported by facts is more likely to arouse a lasting interest in the subject than facts alone.

Then, too, the facts are so meager. There are much experimental data, but a large part of them is controversial. To give the student a single bare fact and leave him without theory is to rob him of all that is worth while in a college course. Theory is the life of any science. Those who have given so much to physics and chemistry during the last quarter of a century are all theorists, Laue, Plank, Bohr, and others. Indeed, these scientists are more than theorists. They are philosophers, and they are so because of their early training in an environment which has long been a center of philosophical reasoning.

To encourage the student to think about his experiments as soon as he learns to perform them is the university point of view. Given the opportunity, college students will handle theory as easily as facts, and with far more interest and enthusiasm.

Three qualities have been striven for in the writing of this book: the avoidance of finality in statement; frequent reminders of the bearing of plant physiology on commonplace experiences; and a presentation as fluent and readable as is consistent with scientific accuracy.

A few bibliographical references are added at the close of each chapter. They are to be regarded simply as the author's choice of an introduction to collateral reading.

The manuscript was read by Professor Howard E. Pulling and Professor Edgar T. Wherry, and portions of it by Dr. Charles Hock; to each I am indebted for many helpful suggestions. Contributions to certain sections have been made by specialists in these fields; to them I am also grateful. In particular am I indebted to Dr. Philip R. White for an account of his work on root culture and root pressure, to Dr. P. Rothemund for reviewing the chapter on photosynthesis, and to Dr. P. W. Zimmerman for assistance in the writing of the chapter on hormones and growth-promoting substances.

The illustrations have been collected from various sources. Many are original drawings and photographs. In all other instances, credit is given to the contributor under the illustration. I am particularly indebted to Miss Helen Ligget for making the majority of the original drawings here published, and to Mr. J. A. O'Brien for Figures 15, 72, and 95.

A few drawings are modifications of old, and well-known illustrations. Where the change is slight, credit is given to the original source, but where the finished drawing bears but little resemblance to any other, no attempt is made to name the several artists.

WILLIAM SEIFRIZ

Philadelphia, Pa.
September, 1938

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CHAPTER I

INTRODUCTION

THE AIM OF PLANT PHYSIOLOGY

Terms and concepts often lose value through definition. "Define your terms" is a frequent admonition; but the physicist cannot define time nor space to his own satisfaction. This, however, does not prevent him from discussing both with profit. The biologist finds it equally difficult to define protoplasm; yet all biologists have a very clear conception of it and find the lack of a precise definition no detriment to their studies. The same is true of physiology. If the present volume is successful in fulfilling its purpose, the reader will know by the time he has reached the last chapter just what physiology is; and that is all that is necessary.

Physiology is the study of living things, or rather the study of the reactions which go on in living things; more specifically, it is the physics and chemistry of life processes. Whatever definition is given of physiology, it should lay emphasis on the fact that it deals with processes. Processes involve change, so physiology is that science which deals with the changes occurring in organisms. Though emphasizing change, physiology cannot ignore structure, for processes are indissociable from structure. The physiologist is, therefore, interested in the mechanism but primarily in what the mechanism does, for physiology is above all a dynamic study, never static.

The physiologist interprets the processes which go on in organisms in terms of physical and chemical laws whenever he can. The known laws may be insufficient, but this must always be regarded as only a temporary deterrent for, though admitting the difficulties, the research worker ever looks forward to the possibility of an answer. Such an attitude replaces the older one expressed by Sachs half a century ago when he said: "We often meet with the view, especially in modern times, that vegetable physiology is virtually only applied physics and chemistry, as though the phenomena of life could be simply deduced from physical and chemical doctrines. This might perhaps be possible if physics and chemistry had no further questions to solve in their own domains, but in fact both are still as far distant from this goal as physiology is from hers." That the phenomena of life cannot always be very *simply* deduced from physical and chemical doctrines is true, but that they cannot be deduced at all is inconsistent with a scientific attitude. It was Pfeffer, pupil of Sachs, who took plant physiology out of the realm of mysticism and based its happenings on the law of causality.

THE SCOPE OF PLANT PHYSIOLOGY

Plant physiology has broadened the scope and aim of all static subjects in botany. Thus, plant classification, though still based primarily on structure, has found additional support from physical and chemical studies of plant grouping.

Starch grains have characteristic markings which may be used for classifying plants according to their starches. Protein analysis has given physiological support to the purely morphological classification of plants. A definite protein relationship between plants has been established by Mez using classical serological methods. Colloidal physics has made its contribution to plant taxonomy through the work of Moyer in which cataphoretic migration and isoelectric points serve as the criteria of plant relationships (page 20). The most encouraging feature of the physiological support given to classical methods of plant classification lies in the close agreement of the several methods.

Plant geography is another descriptive science the scope of which has been broadened through physiological interpretations. Ecology is the study of the relationship between a plant and its environment. Although the subject still remains in part descriptive, much of it involves such physiological relationships as the bearing of climate, transpiration, and soil acidity on distribution. It is a fundamental principle of ecology that an organism is an expression of its environment, and to interpret this involves physiological methods.

The outlook of the older science of cell anatomy has been enlarged through the application of physiology. In distinguishing what is real from what is an artifact, the student working on killed and stained material is aided by supplementary studies on living cells. The function of cell parts has likewise been ascertained through physiological studies of living material.

Genetics, that branch of biology which deals with the laws of heredity, becomes a part of physiology when it deals with the processes of reproduction and evolution.

Experimental pathology has shown such extraordinarily rapid development because of the application of physiological methods of research. The modern pathologist now recognizes the importance of a knowledge of the physiology of the diseased plant and the physiological relationship between parasite and host to a full understanding of his problems.

Agriculture offers the greatest possibilities for the practical application of physiology. Increase in crop yield through the addition of fertilizers, proper handling of the soil, development of plant resistance to drought and frost, advance in time of flowering and fruiting through the shortening of exposure to daylight, are problems in plant physiology.

Soil science is concerned with the geology, physics, and chemistry of the soil. It deals also with problems in the interrelationship between plant and soil, such as deforestation, cultivation, and the wasting of large areas of land. These studies involve, in part, a knowledge of the physiology of the plant. The vast microscopic population of the soil, upon which the successful growing of plants depends, is still another problem which is the mutual interest of soil scientist, agriculturist, and physiologist.

Medical botany includes a number of studies which combine problems in medicine and plant physiology. Certain skin eruptions are due to poisonous plants, and hayfever is produced by the proteins of pollen. Here the physiological relationship between host and toxic plant substance is a problem in both immunology and physiology. Cerebral cystitis is caused by a yeast entering the nasal passages and lodging in the brain; ringworm is due to the fungus, *Penicillium brevicauli:* a complete understanding of these diseases must include a knowledge of the physiology of the plants involved.

Industrial botany offers a great variety of problems which have to do with the physiology of plants. The commercial production of alcohol is accomplished by the activity of yeast cells: the conversion of alcohol into vinegar takes place through the agency of bacteria: the baking of bread and the brewing of beer involve the activities of yeast: the flavors of cheese are due to fungi, the proper culturing of which is of importance. These are all problems in physiology.

The commercial chemist manufactures fertilizers and insecticides. He cannot do this successfully without an accurate knowledge of the reactions of plants to these substances.

The plant physiologist is often called into consultation to settle disputes between farmers and nearby gas or petroleum companies. The farmers claim that their crops are damaged by escaping gas or oil. The physiologist must decide if the abnormal condition of the plant is due to gas, oil, fungus, or some other agent.

Plant physiology is helpful in gaining an accurate knowledge even of such remotely related subjects as sociology, economics, psychology, and philosophy. For example, conditioned reflexes occur in plants.

In setting forth the contributions made by plant physiology to other sciences, the debt which it owes to them should not be forgotten. Without physics and chemistry there would be no physiology worthy of the name.

The problems dealt with on the following pages may be discussed quite aside from their bearing on the other sciences and on the practical affairs of daily life. Utilitarian applications of plant physiology may be kept in mind but they should not be the motive of the research, if fundamental and far-reaching results are to be achieved. Furthermore, the research worker need have no fear that his theoretical results will not find a useful application, for theoretical science and applied science are inseparable; they are one and the same, as the fruit is to the tree.

THE HISTORICAL BACKGROUND OF PLANT PHYSIOLOGY

Theophrastus, who lived in the Fourth Century B. C., wrote on the history and causes of plants. His contributions to botany were not only the most critical and profound of his day, but remained so until modern times. With the sagacity of philosopher and scientist, Theophrastus saw into the reason of things. He realized that the root is more than an anchor for the plant, and that through it "aliment is taken up."

The knowledge of the ancients was forgotten while civilization floundered through the dark ages, from which botany did not emerge until about 1530.

The reawakening of botany was heralded by the publication of a folio by Brunfels, dealing with the classification of plants and their medicinal values. For the next two hundred years botany was almost entirely descriptive. This era was closed by Linnaeus, who epitomized the aim of botany for the two centuries that preceded him. Studies in taxonomic and descriptive botany still play an important part in botanical science, but they no longer dominate investigation as they formerly did.

Stephen Hales, through the publication of his "Vegetable Staticks" in 1727, inaugurated a "new botany," the physiology of plants. Hales was a true experimenter. His work on blood pressure in animals led him to believe that there might be a sap pressure in plants, and he proved its existence with what today would be called a manometer. Because the pressure was upward only, he denied the presence of any circulation in plants comparable to that in animals. With Hales' measurements of root pressure, plant physiology had its beginning. That Hales had the discernment of a true experimenter is shown by his account of an experiment:

> "Having in the 1st chapter seen many proofs of the great quantities of liquor imbibed and perspired by vegetables, I propose in this, to enquire with what force they do imbibe moisture.
>
> "Tho' vegetables (which are inanimate) have not an engine, which, by its alternate dilations and contractions, does in animals forcibly drive the blood through the arteries and veins; yet has nature wonderfully contrived other means, most powerfully to raise and keep in motion the sap, as will in some measure appear by the experiments in this and the following chapter.
>
> "I shall begin with an experiment upon roots. . . .

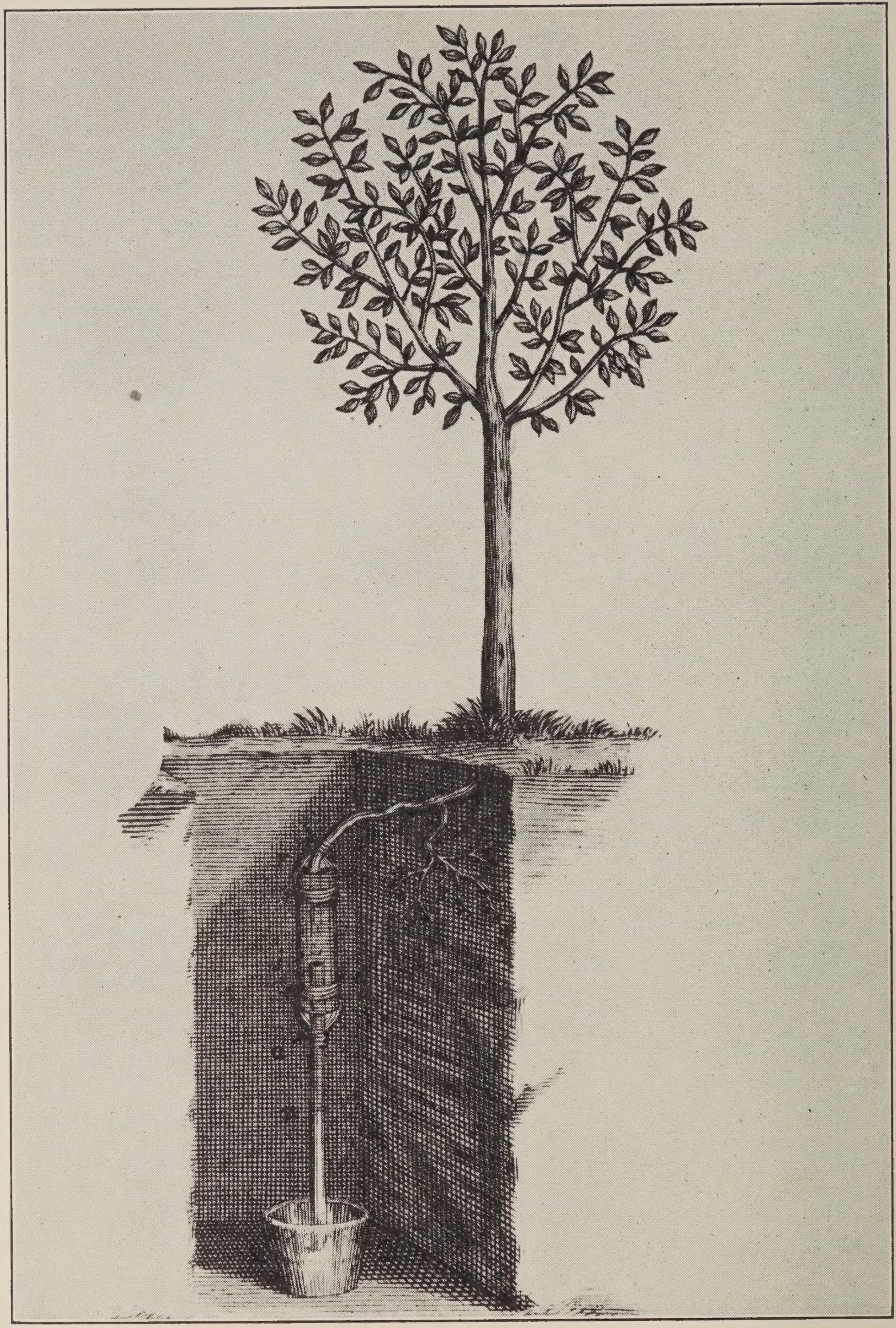

FIG. 1. Stephen Hales' experiment on root pressure.

> "August 13. In the very dry year 1723, I dug down 2 1/2 feet deep to the root of a thriving baking *Pear-tree*, and laying bare a root 1/2 inch diameter *n*. (Fig. 1) I cut off the end of the root at *i*, and put the remaining stump *i n* into the glass tube *d r*, which was an inch in diameter and 8 inches long, cementing it fast at *r*; the lower part of the tube *d z* was 18 inches long, and 1/4 inch diameter in bore.
>
> "Then I turned the lower end of the tube *z* uppermost, and filled it full of water, and then immediately immersed the small end *z* into the cistern of mercury *x*; taking away my finger, which stopped up the end of the tube *z*.
>
> "The root imbibed the water with so much vigor, that in 6 minutes time the mercury was raised up the tube *d z* as high as *z*, viz., 8 inches."

While Hales was the most renowned of the early workers in plant physiology, others made contributions none the less important. There is, for example, the experiment of the medical chemist, van Helmont, in the early years of the Seventeenth Century. He says: "I took an earthen vessel in which I put 200 pounds of soil dried in an oven, then I moistened with rain water and pressed hard into it a shoot of willow weighing 5 pounds. After exactly five years the tree that had grown up weighed 169 pounds and about three ounces. But the vessel had never received anything but rain water or distilled water to moisten the soil when this was necessary, and it remained full of soil, which was still tightly packed and, lest any dust from outside should get into the soil, it was covered with a sheet of iron coated with tin but perforated with many holes. In the end I dried the soil once more and got the same 200 pounds that I started with, less about two ounces. Therefore the 164 pounds of wood, bark, and root, arose from the water alone." Though the deductions of van Helmont are erroneous, his experiment proved that organic matter is produced by plants.

The foregoing investigations were by individual and intrepid workers. They were pioneers who blazed the trail. It is now just a century since plant physiology became a recognized branch of science. How far-reaching have been its influences is to be seen from a single series of events which led uninterruptedly from the casual observation of a physiologist to a fundamental contribution to physical chemistry.

In 1837 Dutrochet observed that when a solution of salt or sugar is separated from pure water by an animal membrane, water diffuses through the membrane more rapidly from the pure-water side than from the solution side, resulting in a rise in the level of the solution and the consequent production of a hydrostatic pressure on the solution side of the membrane. To the movement of water under these conditions, Dutrochet gave the name "osmosis." In the meantime, the botanist, Naegeli, had discovered the selective properties of living plant-cell membranes. Then came the studies of Hugo de Vries, who formulated the principles underlying osmosis and applied them in an effective way to the determination of molecular weights. Next followed the measurements by Pfeffer, in 1877, of the pressures developed in plant cells. These studies resulted in the publication of his classical work, "Osmotische Untersuchungen." To him also is due the credit for bringing the concept of ions into the interpretation of physiological phenomena. The Dutch botanist, de Vries, and his colleague, the physical chemist, van't Hoff, often met and discussed osmosis in plant cells. This mutual interest led van't Hoff to his discovery of the application of the gas laws to solutions. Arrhenius then interpreted the anomalous behavior of electrolytic solutions in terms of his dissociation theory; and physical chemistry had its beginning. Thus did an observation on the physiological behavior of plant cells contribute to the birth of a new branch of science.

In the foregoing brief account of the development of one line of physiological study which made an important contribution to physical chemistry, the greatest years of plant physiology have been touched upon, and, with a few exceptions, the greatest names associated with it have been mentioned.

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CHAPTER II

THE COLLOIDAL STATE

Knowledge of the *colloidal state* of matter serves as a basis for the better interpretation and understanding of the physiology of organisms.

Jellies are colloidal systems, and protoplasm in its finer structure is a jelly. Certain properties of jellies are very typical of biological material; thus, the viscous and slimy substances common in the living world, such as plant gums, are jellies. *Emulsions* (Fig. 2) are colloidal systems, and protoplasm, in its coarser structure, is an emulsion; so also is latex, a fluid present in certain plants. If there are emulsions and jellies in the living plant, then only through a knowledge of them can the physiological problems which concern them be handled.

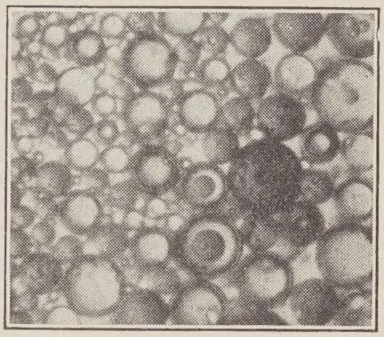

FIG. 2. An emulsion of oil in water. Note that some of the oil droplets contain smaller globules, i.e., are themselves emulsions.

A definition or characterization of the colloidal state is given with difficulty until it is made clear that in spite of some few similarities there are two main groups of colloidal systems which really have very little in common. The few similarities which exist are far outweighed by the fundamental dissimilarities between the two groups. Still, both have become known as *colloids*, and nothing is to be gained just now by any effort to exclude one or the other from the colloidal state of matter.

The two groups of colloidal systems are the glue-like ones, which include gelatine, agar, gums, and white of egg; and the suspensions (Fig. 3) which include turbid water, smoke, foam, and mist. There seems to be little in common between these two kinds or states of matter, yet there are several properties which have caused them to be incorporated into the one class "colloids." Chief among these is the property which gave rise to the distinction between *colloids* and *crystalloids*.

The English chemist, Thomas Graham, discovered (or rediscovered), in 1861, that salts, sugars, and like substances which go readily into aqueous solution pass through parchment membranes, whereas substances such as gelatine and glue, which do not go readily into solution, do not pass through these membranes. The former, Graham called *crystalloids*, as they all crystallize readily; and the latter he called *colloids*, meaning "glue-like" (Gr. *kolla*, glue; *eidos*, semblance), because substances such as glue are typical of them.

The study of "glue-like" matter and the related fine suspensions is the field of *colloidal chemistry*. It is the study of a *state*, and not a *kind* of matter; thus, chemically, gelatine is a protein; only in respect to its physical properties is it a colloid.

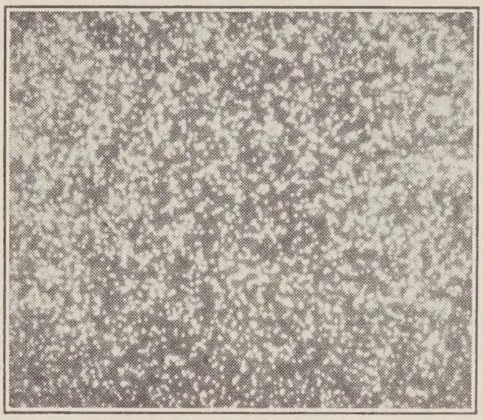

FIG. 3. Sapphirine glass seen against a dark-field produced by the lateral illumination of a slit ultramicroscope: the colloidal particles in the glass are gold (Photograph Carl Zeiss, Jena.)

Pioneers in every new science grope blindly in the dark, seeking facts out of which to build a solid foundation. Many of the building stones will prove inadequate and must be discarded. Colloid chemistry is still a young science; its definitions, criteria, and rules will undergo much modification. Many "colloids" have now been obtained in the crystalline state, and are in this sense crystalloids, and many "colloids" pass through membranes such as parchment. However, the fundamental concepts of the colloidal state still hold.

If a solution containing both a colloid and a crystalloid, say a protein and a salt, is put into a sac of parchment paper and the sac immersed in water, the crystalloid will diffuse out and the colloid will remain within, provided the porosity of the membrane is fine enough. This process of separation, which is sometimes used to purify colloids, is known as *dialysis* (Fig. 4).

While the properties of colloidal suspensions, particularly the emulsions, are of interest and value to the biologist, it is the gel-forming colloids which are of greatest significance in physiology. These latter include such important plant and animal products as gum acacia, latex (rubber), agar, resin, albumin, gelatine, casein, blood, charcoal, porous clay, and protoplasm. The chief characteristic of these colloids is that they all form jellies.

When a solution of gelatine is hot, it is fluid; when it cools, it *sets* or *gelatinizes*, if sufficiently concentrated. To the liquid condition, Graham gave the name *sol*, from *sol*ution, and to the firm or jelly condition he gave the name *gel*, from *gel*atine.

Some jellies may, under proper conditions, be readily reversed by simply reversing the process which brought about gelatinization. Cooling a hot solution of gelatine causes it to set; heating brings about reversal. There are many gels, however, which cannot be reversed so simply; thus, boiled albumin is not reversed by cooling. Irreversible gels are *coagula*, and the process of their solidification is *coagulation*.

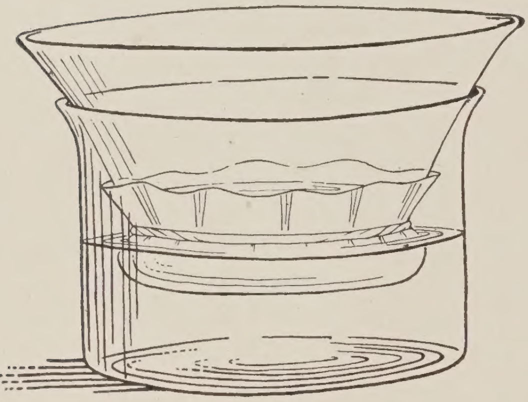

FIG. 4. Thomas Graham's dialyzer.

There has been much confusion in regard to the use of the word "gel," some preferring to limit it to *coagula*, e.g., the silica gel, whereas others, following Graham, prefer to regard jellies as gels. Graham coined the word "gel" from the first three letters of gelatine; he must, therefore, have had hydrated gelatine in mind. Gelatine is not a coagulum but a jelly. Confusion is avoided by taking a broad view of the matter and retaining "gel" as a group term to include both *reversible gels* or jellies and *irreversible gels* or coagula.

Difficulties in nomenclature are not serious in themselves, but they may lead to fundamentally fallacious concepts; thus, it matters little whether a gel is a jelly or a coagulum, but it is of great importance to know in what way a coagulum differs from a jelly. Jellies, e.g., gelatine, swell in water, are elastic, and non-porous; coagula, e.g., pumice stone, take up water but do not swell, are relatively inelastic, and are porous. It is the porosity of coagula which makes them good adsorbents, e.g., charcoal.

The two major classes of colloids have been variously named, with emphasis on one property or another. Gels or jellies, such as gelatine and protoplasm, take up water with avidity and are therefore said to be *hydrophilic* (*hydro* = water; *philic* = loving), or *lyophilic* (solution-loving). The suspension colloids, such as emulsions and smoke, show no affinity for water, and are therefore said to be *hydrophobic* (*hydro* = water; *phobic* = hating), or *lyophobic* (solution-hating).

In addition to these names, the two major groups of colloids have received numerous other designations, among which are *suspensoids* for the suspension colloids and *emulsoids* for the gel-forming colloids. This latter term has been the cause of much misunderstanding in colloidal chemistry and physiology and should be dropped. It is a misnomer brought into colloidal chemistry when the science was young; it is much used, but often wrongly. It originally included both gels and emulsions, systems which have little in common. It is now used solely to indicate gels; but as gels are not liquid-liquid systems, they are obviously not "emulsoids," that is to say, not "emulsion-like."

Colloidal systems such as smoke, clouds, and fine silt in water consist of particles of one kind of matter—carbon, water, and quartz in the examples just cited—finely dispersed and suspended in another kind of matter. This fact and that other one, dialysis, on the basis of which Graham distinguished colloids from crystalloids, have led to the particle concept which has dominated colloidal chemistry from the beginning. The presence of particles in the lyophobic or suspension colloids is readily demonstrated; the Tyndall phenomenon (page 17) does this. Certain gels, such as porous clay and meerschaum, are without question formed of adhering particles. But certain other lyophilic or gel-forming colloids may not be granular; particles, in the sense of molecular aggregates, may be lacking. It has in the past been assumed, however, that all gels are built of submicroscopic particles to which the special name *micelle* has been given (Latin = *micella, micellae;* English = micelle, micelles). Colloids may, therefore, be arbitrarily defined as systems built of micelles or particles above molecular dimensions but below microscopic visibility. The colloidal suspensions thus include all forms of matter wherein particles larger than molecules are in permanent suspension. The scattered particles are the *dispersed* or *discontinuous* phase, and the medium in which they are suspended is the *dispersion medium* or *continuous phase.*

If the conditions of colloidality are satisfied when one kind of matter (gas, liquid, or solid) is finely divided and permanently suspended in another kind of matter, then there should be nine types of suspension colloids. All but one of these exist, the exception being gas dispersed in gas, which is always a molecular and never a colloidal dispersion. The eight types of colloidal suspensions, with examples, are:

Type	Example	Dispersed Phase	Dispersion Medium
Gas in liquid	foam	air	water
Gas in solid	porous clay	air	clay
Liquid in gas	mist	water	air
Liquid in liquid	emulsions	oil	water
Liquid in solid	pearl	water	calcium carbonate
Solid in gas	blue smoke	carbon	air
Solid in liquid	colloidal silver	silver	water
Solid in solid	black diamond	graphite	crystalline diamond

In order to grasp clearly the magnitude of colloidal dimensions it is necessary to know the scale which serves as a measure. The millionth part of a meter (the thousandth part of a millimeter) is the unit of microscopic dimensions; it is called a *micron* and has the symbol μ. The thousandth part of a micron is an mμ and the millionth part of a μ is a $\mu\mu$. Cells range from 0.15 mm to 1 μ (0.001 mm); colloidal particles range from 0.1 μ to 10 mμ. Molecules range from 46 $\mu\mu$ (water) to about 2.5 mμ (protein). To this scale, each member of which (mm, μ, mμ, $\mu\mu$) is a thousand times the one below it, may be added the *Ångström Unit*, A. U., used chiefly in indicating the wave length of light and the dimensions of atoms. It is 0.1 mμ and therefore 100 $\mu\mu$. The light waves of the visible spectrum are around 7500 A. U. long at the red end and 4000 A. U. long at the violet end.

Colloidal particles are usually near or below the limit of microscopic visibility; for this reason the colloidal state has been picturesquely described as the "twilight zone of matter." Colloidal particles are also above molecular dimensions for they are aggregates of molecules. Consequently, the upper and lower limits of the colloidal world may be arbitrarily placed at 0.1 μ (or 100 mμ), and 10 mμ. The former dimension is at the limit of microscopic visibility, and the latter is four times the diameter of a large protein molecule. However, colloidal particles as small as 3 mμ have been detected, and some colloidal systems such as emulsions and, in particular, foams have particles far above 0.1 μ.

Here, as in all new sciences, arbitrary terms and concepts creep in which are eliminated only with time and increase in knowledge. Permanent suspension and size of particle are relative terms. Milk creams over night, yet it is an emulsion and as such possesses colloidal properties, even though relatively unstable. Smoke settles, yet for a time it is colloidal. The bubbles in foams may be very large, far above microscopic visibility, yet foams are colloidal. If such systems are of the colloidal world, there must be a property common to them and to fine dispersions. That property is the high proportion of surface to volume.

Of all the features of colloids, the tremendous surface exposed is the most fundamental. Surface is more important than particle size, because, although the latter is usually responsible for the great increase in surface, it need not exist in order to have great surface. How vast the increase in surface may be is illustrated by the repeated subdivision of a cube.

Size of Cube, Edge in Centimeters	Number of Cubes	Total Surface in Square Centimeters
1.	1	6
0.1	1,000	60
0.01	1,000,000 (10^6)	600
0.001	10^9	6,000
0.0001	10^{12}	60,000
0.00001	10^{15}	600,000

Still another example is that of 1 cc of water which if sprayed into droplets 0.01 μ (0.000001 cm) in diameter attains a total area of 6,000,000 sq cm.

That surface rather than particle size is the distinguishing feature of the colloidal world is evident in systems such as baked porous clay, dried silica gel, and charcoal in which both phases are continuous, the solid phase being of clay, silica, or charcoal, and the gaseous phase of air. Such gels are, like sponges, finely porous, solid matter shot through with minute capillaries. Meerschaum and spongy (colloidal) platinum are similar systems. Alloys are colloidal; increase in surface alone, and not particles, distinguish them. Spongy platinum illustrates how significant an increase in surface may be from the energy point of view. Metallic (solid) platinum is relatively inert, but spongy (colloidal) platinum is an excellent catalyst or activator of reactions.

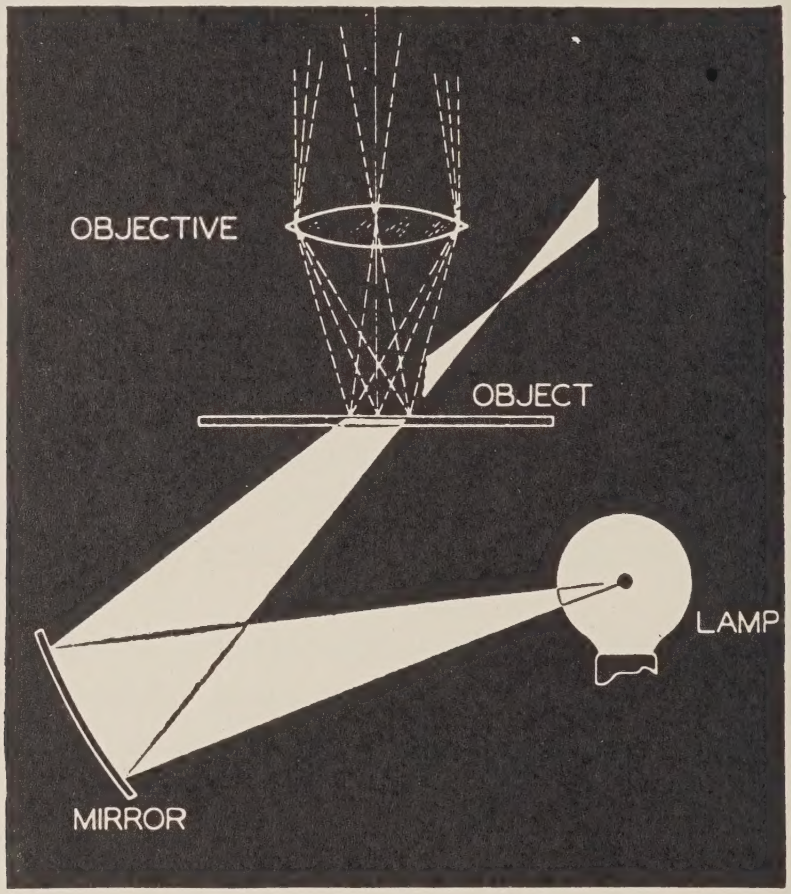

Fig. 5. A simple dark-field optical system. (From Bausch & Lomb Optical Co.)

If a beam of light is allowed to pass through a colloidal suspension and the suspension is viewed against a dark background at right angles to the beam, a brilliant cone of light will be seen. One can readily obtain this effect by blowing tobacco smoke into a beam of light. The phenomenon was discovered by Faraday and studied by Tyndall, so it is known as the Faraday-Tyndall effect, or more commonly as the *Tyndall cone.* It owes its existence to the scattering of light by colloidal particles. The light is not reflected, for the particles are too small to act as mirrors; they merely interfere and bring about diffraction.

The Tyndall cone is the gross effect of the scattering of light by many particles in colloidal suspension. If such a suspension is viewed against a dark background, the individual particles appear to be visible in that they are "seen" as centers of bursts of light (Fig. 3). An optical equipment for viewing colloidal particles with lateral illumination against a black background is known as an *ultramicroscope.* In its simplest form it consists of an arrangement of parts such as that pictured in Fig. 5. A more convenient and efficient form of dark-field illuminator is the *cardioid condenser* (Fig. 6).

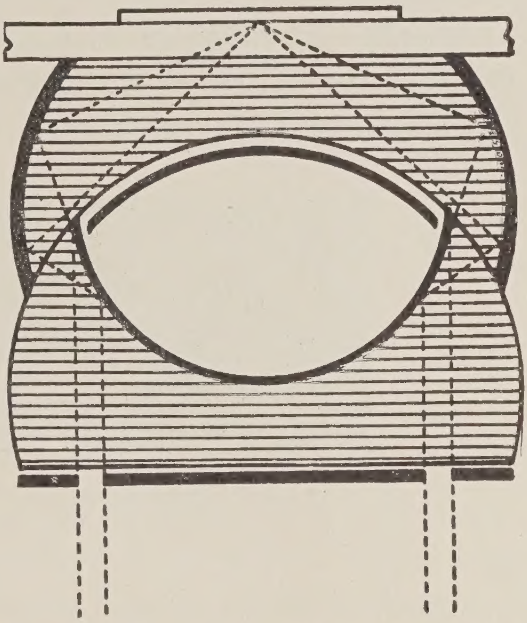

FIG. 6. The path of light through a cardioid dark-field condenser.

Results obtained with dark-field illumination differ in their value. Often, real structural units are so confused with diffraction phenomena that it is impossible to interpret the picture. Occasionally, however, a colloidal structure is revealed that is sharp, real, free of diffraction lines, and not visible with direct light. The sculpturing on the silicious walls of diatoms is vividly revealed by dark-field illumination (Fig. 7); so also the colloidal structure of the cellulose wall of plant cells and coal (Fig. 50). Dark-field illumination is like the surgeon's knife; dangerous in the hands of the untrained, but useful in the hands of the competent worker.

One of the most characteristic properties of colloidal particles, and a very significant one, is their incessant helter-skelter motion known as *Brownian movement.* The movement is due to the bombardment of colloidal particles by the surrounding invisible molecules. Brownian movement ceases when particles are above 4 μ in diameter, if suspended in water. As the amplitude of Brownian movement bears a definite relationship to the consistency of the medium—particles which are active in water may be quiet in glycerine—the presence of the motion, and in particular, its amplitude, are measures of the consistency of the dispersion medium. The method has been used for determining the viscosity of protoplasm.

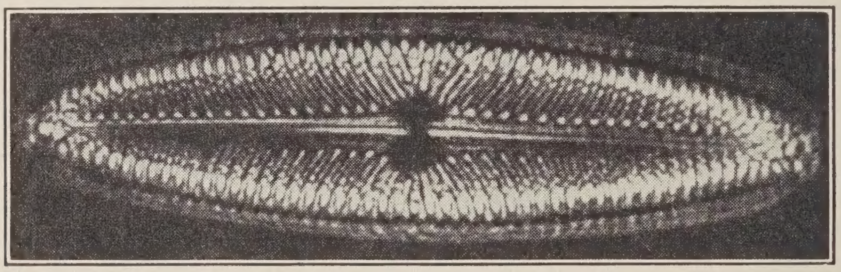

FIG. 7. The diatom, Navicula, in dark-field: x 2160. (Photograph, Charles Spierer.)

Another fundamental property of colloidal particles is their electric charge or potential. All particles in any one suspension are of the same sign as to charge, all positive or all negative. Colloidal particles of platinum, gold, and silver are negative; lead, iron, and copper are positive. The presence of a charge can be proved in a number of ways. If colloidal particles are put in an electric field, they will migrate to one pole or the other depending upon the sign of their charge. Their movement under these conditions is known as *cataphoresis.* Cataphoretic migration is not only characteristic of colloidal particles but of living cells as well, and of particles within cells.

If colloidal suspensions of opposite charge are intermixed, they will mutually precipitate each other. This experiment shows that the stability of colloids, i.e., the fact that the particles remain in suspension, is related to their potential. The potential on colloidal particles is of the order of 50 millivolts (0.050 volts). When the potential is reduced, theoretically to zero, the particles no longer migrate in an electric field. They collide, adhere, and fall. The point of no migration is known as the *isoelectric point*; usually, though not necessarily, it is expressed in terms of acidity or *p*H. Actually, precipitation occurs in practice above zero potential, at a value known as the *critical potential.*

Colloids may be precipitated by colloids of the opposite sign or by electrolytes. Positively charged particles are brought down by anions such as NO_3^- and PO_4^{---}, and negatively charged particles by cations such as K^+, Ca^{++}, and Al^{+++}. This phenomenon is nicely illustrated in the formation of deltas. When the muddy waters of the Nile or the Mississippi reach the sea, the fine clay particles in suspension are brought down by the salts of the sea and deltas result.

Under proper conditions, the sign of the charge on colloidal particles can be reversed. In the case of metals, reversal is accomplished by the addition of ions of high valence, e.g., Al^{+++}. Proteins are more readily reversed, in many cases simply by a change in acidity owing to the *amphoteric* character of proteins (page 183).

The stability of colloidally dispersed particles is due to their electric potential and their Brownian movement; Brownian movement prevents settling, whereas charge through mutual repulsion prevents coalescence.

When their surface potential is reduced to zero, Brownian movement still functions in maintaining stability until the particles come into contact, when they adhere, aggregate, and settle.

In proteins and other lyophilic dispersions, still another factor enters into stability, namely hydration. If the charges on protein particles in suspension are neutralized, the particles may not precipitate. The water film with which they are surrounded keeps them from adhering when they collide. If they are dehydrated, e.g., with alcohol, as well as de-charged, they will coalesce and fall.

Related to the cataphoretic migration of particles in an electric field is the movement of water through capillaries under the influence of an electric potential. This phenomenon is known as *electroendosmosis*. It will take place through a block of wood —the xylem elements and the pores in the cellulose walls serve as capillaries.

The electric properties of colloidal particles are also typical of living cells. Bacteria, yeast, spores, sperm, and practically all cells migrate in an electric field. Cells are negatively charged when in their natural environment. They coalesce when their interfacial potential is reduced to the critical potential, which is about 10 millivolts for bacteria; they may thus be precipitated with electrolytes. The potential and isoelectric point are so characteristic of organic colloidal suspensions that Moyer found he could identify various species of spurges (*Euphorbia*) by the mobility curves and isoelectric points of their respective latex particles. Thus does a wholly colloidal method of approach find application not only in physiology but in taxonomy.

Biologically the solid suspensions have little in common with protoplasm. The liquid suspensions, or emulsions, and the jellies are of greater physiological significance. To be sure, certain properties of solid suspensions, such as migration in an electric field, are possessed by living matter, but the resemblance goes no farther. Superficially protoplasm is an emulsion; fundamentally, it is a jelly.

Emulsions consist of two immiscible phases, usually oil and water, one of which is finely dispersed in the other. The naturally occurring common emulsions are of oil scattered as minute globules in water. Milk and medicinal emulsions are of the *oil-in-water* type, whereas cold cream is a *water-in-oil* emulsion. Most emulsions can be reversed from the one type to the other, some very readily so, others not, which may be merely a matter of finding the correct electrolyte (salt or base) for doing it.

An emulsion of pure oil in pure water is difficult to make and is usually stable only if the dispersed phase (oil) is less than one hundredth part of the dispersion medium. In order to make emulsions of equal parts of oil and water, a third phase, the *emulsifier* or *stabilizer*, must be present. Any gel-forming colloid, such as gelatine or soap, is a good stabilizer. It functions by forming a membrane around the dispersed globules. Thus, in milk the scattered droplets of butterfat are surrounded by delicate membranes of protein material, probably casein. The stabilizing membrane functions as a protective layer by preventing coalescence when the globules collide.

Hypotheses of protoplasmic behavior have been based on the behavior of emulsions (page 125). There is much to be said against such hypotheses, yet it is nevertheless an interesting fact that the proportion of sodium and calcium (50 : 1) which will keep an emulsion at the reversal or breaking point is the same as that which occurs in sea-water, blood, and milk. Emulsions play their part in protoplasmic behavior, chiefly as a source of food supply and as the seat of surface reactions.

But it is the gel-forming systems which find the greatest applicability to problems in plant chemistry and plant physics. The high viscosity of jellies distinguishes them from colloidal suspensions. Like jellies, most plant products are exceedingly viscous. Also typical of jellies is their extraordinary powers of imbibition. The taking up or imbibing of water by gels is a phenomenon of common experience; thus, wood swells in water because of *imbibition*. The *imbibition pressure* developed thereby may be very great. The ancient Egyptians quarried stone by wetting wooden wedges which had been driven into the stone when dry. The imbibition pressures developed by various gel-forming substances are of much value to the plant. The bursting of the tough coat of seeds at germination is accomplished by imbibition pressure.

Jellies are elastic. This property tells more of the probable ultramicroscopic structure of gelatine, cellulose, and protoplasm than any other. It indicates continuity in structure.

Coagulation is a property common to many non-living and living colloidal solutions, such as albumin, casein, blood, and protoplasm.

Thixotropy is an extraordinary property of some gels; it resembles but is distinct from liquefaction, setting, and coagulation as ordinarily experienced. Certain gels, such as that of ferric hydroxide, may be liquefied, or reduced to the sol state, simply by mechanical agitation, following which they very quickly solidify, and may then be again reduced to a sol by shaking. This sudden collapse of a gel followed by resetting is known as thixotropy. Protoplasm is thixotropic. The complete collapse of a cell such as an echinoderm egg, when in mid-mitosis, may be brought about simply by the application of a sudden mechanical shock. All vestige of the former mitotic figure disappears. The sudden reversible changes in viscosity which protoplasm frequently undergoes are probably due to thixotropic behavior.

Thus is it evident that the physiologist cannot progress far in his studies of the living plant without a substantial knowledge of colloidal systems.

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CHAPTER III

THE SOIL

The importance of soil conditions to daily life is seldom fully realized. Most people are aware of the need of plowing the soil and occasionally adding fertilizers, but there knowledge, and unfortunately often interest, end. The soil is the provider of all food for mankind and as such becomes the basis of civilization.

Life probably originated in the soil, and life is maintained by the soil, air being the only additional source of a primary vital element. Well does soil deserve the name "Mother Earth," for here all vegetable life is reared, from which all animal life is nurtured.

Fundamentally, soil is weathered rock, but this, like most definitions, is inadequate. To be capable of supporting higher plant life, a soil must contain not only mineral matter, but also decayed organic matter, water, air, and microorganisms.

Soil is that part of the earth's crust which supports plant life; it extends but a few feet below the surface. Roots may at times penetrate much farther, in deserts going to 25 or even 50 feet down to water; but they are then far beyond what can be strictly termed soil.

The composition of soil is primarily determined by the composition of the earth's crust. Soil is only the uppermost layer of this crust. The earth's crust or *lithosphere* is some 40 or 50 miles thick and is made up largely of oxides of aluminum and silicon. The core of the earth consists mostly of the heavier elements, iron and nickel, in a free, non-oxidized state.

Soil constituents belong mainly to one of four groups, other components being present only in minor amounts. These groups are: silica; sesquioxides of iron and aluminum; oxides of the strongly basic elements, calcium, magnesium, potassium, and sodium; and organic carbon compounds. The proportions of these ingredients will vary considerably in different soils.

The foregoing is a chemical classification of soil constituents. Physically, soil components consist of *sand*, *silt,* and *clay*. Mixtures of these in various proportions occur, resulting in soils of intermediate textures, e.g., sandy clay. Other terms frequently employed in soil technology are *loam*, which is a mixture of fine sand, clay, and organic matter; and *loess*, which is a very fine and homogeneous sediment, sometimes more or less fossiliferous. Loess is usually of aeolian origin (i.e., wind-laid). It covers large areas in the midland United States, where it forms excellent soils.

The minerals from which soil is formed include *feldspar, quartz, calcite, dolomite, hornblende, mica, magnetite, garnet, apatite, hematite, gypsum,* etc. In these minerals occur the elements necessary to plant life—potassium in feldspar and mica, phosphorus in apatite, and so on.

Surface soil has often undergone so great a change, however, that none of the foregoing minerals may exist as such within it; yet if the composition of the original rock is compared with the decomposition products, it is seen that, although the ratios are different, the same elements are present. The following tabulation illustrates this:

ANALYSES OF ROCKS AND THEIR DECOMPOSITION PRODUCTS

(From F. W. Clarke)

OXIDE	MICACEOUS GNEISS ALBEMARLE CO., VA.	
	Original Rock	Derived Soil
SiO_2	60.69	45.31
Al_2O_3	16.89	26.55
Fe_2O_3	9.06	12.18
MgO	1.06	0.40
CaO	4.44	trace
Na_2O	2.82	0.22
K_2O	4.25	1.10
P_2O_5	0.25	0.47

From the standpoint of mechanical composition, a soil analysis runs somewhat like the following:

Maryland Collington Loam

(From C. F. Marbut and R. S. Holmes)

	Per Cent		Per Cent
Fine gravel	0.2	Very fine sand	12.1
Coarse sand	3.7	Silt	17.9
Medium sand	6.2	Clay	8.0
Fine sand	51.8		

The coarser fractions of which the percentages are given in the above table form, as it were, the framework of the soil; but the finer fractions, especially the clay or colloidal portions, are most important, since it is from them that roots extract most of the nutrient elements.

Incorporated with the mineral constituents of soil are the remains of plants and animals which, in the form of partially decomposed organic matter, are an important part of the soil from the point of view of plant growth, and, correspondingly, of agriculture. The partially decomposed organic remains constitute what is known as soil *humus*. The total organic matter in the soil is generally referred to as humus but there is lack of agreement in the use of the term. Humus is also defined as that portion of the soil organic content which is soluble in alkalies. On the basis of this definition, a great deal of the organic matter in the soil would not be humus. Humus contains at least some two dozen different compounds belonging to nine chemical groups.

"Humic acids" and "humates" have been postulated but their existence is questioned. The organic matter of the soil may be present in the form of complex colloidal systems, rather than as definite chemical compounds.

Soil composition is often partly determined by climate. For example, soils in arid regions are strongly basic, for bases tend to accumulate when evaporation exceeds rainfall. The most ancient civilizations were built on arid soils.

Soil Profile. The foregoing discussion has had to do primarily with surface soil. Beneath it lies the *subsoil*. Soil systematists regard "subsoil" as a loose and non-technical term.

According to modern views, the soil *profile* consists of three *horizons*. The upper or A horizon is commonly referred to as surface soil. It is usually coarser in texture than the lower, and frequently, though not always, contains the larger amount of organic matter. The middle or B horizon is what is commonly known as subsoil. It is transitional in character between the surface soil above and the substratum below. The lowest or C horizon comprises the more or less weathered parent material, from which the soil was originally derived.

Hardpan. Hardpan is a dense and hardened layer in the subsoil which is usually impermeable to water and to roots. The slowing up of the downward percolation of surface water by a finely granular layer causes the concentration of cementing materials present in solution. Various compounds may function as cements, but most are calcareous in nature.

Soil Water. Soils are supplied with water from above by rain and from below by subterranean streams and *ground water*, the surface of which is the *water-table.* The supply of rain water is occasional, whereas subterranean water is permanent, unless the water-table falls beyond the reach of roots. The amount of water taken in by the soil from rain is in large measure determined by the character of the soil surface. A sun-baked clay will absorb little rain; loose soil will absorb a great deal.

The reliance of the plant on soil moisture in certain localities is evident in the great variability of the rainfall. In some desert regions rainfall may be a mere fraction of an inch—often none at all for several years, as in parts of western South America, and the Arabian peninsula—or it may be as much as 805 inches (67 feet)—as at Cherapundji, India where the average is 368 inches. In Louisiana the average annual rainfall is 56 inches; in Pennsylvania it is 42 inches; in western Kansas it is 19 inches; and in Nevada but 9 inches. Twenty inches a year is the limit below which most common crops cannot be grown without irrigation.

The water-holding and water-conducting powers of soil are important factors in supplying the plant with soil moisture. Frequent rain is of little value if the soil will not hold water, and a nearby water-table is of little use if the soil will not conduct water. The power of the soil to hold water is determined primarily by texture, that is to say, by the size of the grains and the capillaries between them. Surface tension, capillarity, adsorption, and imbibition all join in transferring water from wet regions to drier ones. The smaller the capillaries in soil the greater the height to which water will ascend.

Where the water content of soil at the surface is low, and that of the air is high, water will be taken in by the soil through absorption. Water so taken in is known as *hygroscopic water.* Summer fogs in arid regions in this manner contribute much to the water requirements of plants. Whether water is taken in from the air or given off will depend upon the relative water content of the soil and the air, and also on the texture of the soil. Sand will evaporate water faster than clay even when both have the same water content on a weight or volume basis.

The Soil Atmosphere. One of the first requisites for good plant growth is well aerated soil, for roots need oxygen for respiration. Porosity is an important quality in connection with soil aeration. A loose, friable soil assures good aeration as well as ready penetration of roots and ample capillary water. The relationship between oxygen content of the soil and plant growth depends in part upon the plant; thus, soil close under a dense sod of grass is likely to be deficient in oxygen. Under these circumstances, certain weeds, the roots of which go deeper than those of grass, cannot survive. Upon this principle depends the success of a lawn free from weeds.

The oxygen content of the soil is partly in gaseous and partly in dissolved form. Aquatic plants rely solely on dissolved oxygen for the respiration of the cells in their roots. Some plants growing in swamps and bogs where the dissolved oxygen is insufficient resort to special devices for obtaining atmospheric oxygen; such are the *pneumatodes* or breathing-knees of the swamp cypress, *Taxodium distichum.*

Soil Temperature. Soil temperature is dependent upon locality, whether high or low land, arctic or tropic. It is also dependent upon soil depth. The average temperature at one locality (Brussels) was 7°C at 3 feet, 13°C at 15 feet, and 17°C at 75 feet; and the annual range at these depths was 10°, 5°, and 0°C, respectively. Soil is thus warmer the greater the depth, except, of course, for the top inch or two in summer. Soil in temperate regions does not freeze at depths much greater than 2 feet. In the arctic, soil may be frozen to great depths; thus, in North Siberia a reading of $-1°C$ was obtained at 350 feet, all the water being, correspondingly, in the form of solid ice.

The temperature of the soil at the surface is dependent upon the physical properties of the soil. Color is a factor; black soils absorb heat more readily than light-colored soils. The character of the surface also influences the amount of heat absorbed.

The success of plant growth is determined to some extent by soil temperature, but plants tolerate a considerable range. Air temperature, because of its much greater variation, is likely to cause damage sooner than soil temperature; however, soil temperature may determine the type of vegetation. Particularly in spring is it important in influencing the success of a crop.

Acidity. When physiologists learned that it was not the replaceable hydrogen in acids to which organisms were sensitive, but the free, electrified hydrogen or *hydrogen ions*, work on soil acidity became very active (page 100). The acidity of the soil influences plant distribution, equilibria within the soil, the availability of soil nutrients, etc. The constancy with which soils hold their *p*H value differs in different soils. Some are well buffered with an aluminum silicate complex, and others not; the former maintain a fairly even acidity value.

Olof Arrhenius, son of Svante Arrhenius, the author of the dissociation theory, has aroused much interest and confidence in acidity as applied to agriculture in his country and in Germany, so much so that a wide-awake north European farmer carefully avoids planting neutral-soil plants on an acid soil, neutralization of acidity by liming being less practiced there than in the United States. Sugar beets have their highest yield in sugar content on neutral soil. Farmers, therefore, are discouraged from risking a crop of sugar beets where the reaction is acid (*p*H 6) or alkaline (*p*H 8). In general, this is a good policy, but the reason is not always the same. For example, a plant may require its nitrogen to be in a certain form; and acidity often governs the form in which the nitrogen occurs. If, however, it is possible to furnish nitrogen in the desired form, then the plant will be indifferent to acidity within limits. Acid-soil plants, such as rhododendron and other members of the Heath family, prefer their nitrogen as ammonia or amines, whereas neutral-soil plants, such as most ordinary garden and crop plants, prefer theirs as the nitrate ion.

There is a tradition among farmers that moss and sorrel, the red-flowered *Rumex acetosella* and yellow-flowered *Oxalis stricta*, are a sign that the soil is "sour." The studies of Wherry, involving actual tests of hydrogen-ion concentration, show, however, that these plants do not correlate with high acidity at all. The soil reaction may be alkaline (to pH 8) even where the growth of sorrel is luxuriant. Actually, the appearance of these weeds indicates sterility, not acidity. The fact that the addition of lime, an alkali, improves "sour" soil would seem to indicate that acidity is a factor, but the benefit of lime is not always that of a neutralizer; it also adds calcium and carbonate ion, which increase the activity of soil organisms such as the bacterium, *Azotobacter* (page 168). Correspondingly, a greater production of nitrates results, and nitrates are fertilizers which augment plant growth. The problem, therefore, which seemed to be one of acidity, turned out to be one of nutrition. However, there are plants, such as bluets (*Houstonia coerulea*) which rarely thrive on neutral soil and usually appear in a field where an acid condition is beginning to develop owing to depletion of the surface alkalies through leaching by rain.

Leaching. Drainage waters carry important minerals from the soil. The process is known as *leaching*. Lime is the compound most abundantly leached out; magnesia is next; then common salt; and then potash. Leaching may become a serious problem in soil fertility, but it is not quite as serious as would at first appear. Of all the compounds leached out, lime alone is required by the plant in any quantity. The others are either not needed by the plant (e.g., sodium chloride) or are needed in very small quantities.

Fertilizers. Deficiency in the mineral content of a soil is met by man through the addition of fertilizers. The choice of a fertilizer is made by knowing, first, the needs of the plant and, second, the deficiencies of the soil. Leading ingredients of plant mineral requirements are phosphoric acid, potash, combined nitrogen, and lime. Farmers often give lime first place among fertilizers. That this is in part justified is evident in the greater luxuriance of crop growth on calcareous soils, as compared with that on adjacent non-calcareous ones. The blue-grass region of Kentucky is underlaid with calcareous formations. "Lime country is rich country."

Two obvious benefits of lime are increase in alkalinity and addition of calcium. But calcium-containing fertilizers accomplish more than this. In particular, the calcium ion they supply becomes adsorbed on the surface of soil-colloid particles, keeping them stabilized and making some of the other nutrient elements they hold more readily available to plants.

As for the other three fertilizers which supply most of a plant's mineral requirements, phosphoric acid, potash, and nitrates, the first may be added as phosphate, prepared from phosphate-containing minerals or from bones, the "bone earth" of which yields superphosphate. Bone meal supplies both phosphoric acid and nitrogen.

Potash may be extracted from feldspar and other silicate minerals. Formerly, commercial potashes were almost entirely prepared from plant ashes. The discovery of potassium salt beds in Prussia and Saxony led to the development of a great industry in these regions, which now supplies almost the entire needs of the world in potash for industrial and agricultural purposes.

The soil is itself a laboratory for the production of the nitrates from which many plants derive their nitrogen. The process is known as the *nitrogen cycle* (page 167). If the soil supply of nitrogen is inadequate, this element may be added in the form of Chile saltpeter, a mineral which was for years the chief source of commercial nitrate fertilizers; various compounds made directly from atmospheric nitrogen are now also available for this purpose.

One of the oldest fertilizers, and formerly for many farmers the only one, is barnyard manure. It is still an excellent fertilizer, but it is not adequate. Decreasing crop yields, even with ample manure fertilizing, led chemists, among whom Liebig was foremost, to study the problem of plant requirements. As a result of their investigations, mineral fertilizers have almost wholly replaced manure. The efficacy of stable manure lies not only in its mineral content, which is not particularly great, but in its effect on the physical properties of soil, on its water-holding power, and on its bacterial activity. Manure increases the number of bacteria by supplying appropriate food for them.

Soil Organisms. Plant and animal life beneath the soil surface is far more abundant and significant than usually appreciated. Bacteria are active in the nitrogen cycle and in the decomposition of cellulose, urea, and other complex organic substances. Still other organisms are engaged in loosening and aerating the soil.

Fungi play a prominent role among soil organisms. Bacteria are foremost among them, but others, for example, actinomycetes and mycorrhizal fungi (page 276), are important.

Chlorophyll-bearing microscopic plants are also numerous in soils; among them is the blue-green alga, *Nostoc.* They occur in large quantities and have great vitality, but their role in the life of higher plants is unknown.

Animals below the soil help in various ways in bringing about decay, in the formation of humus, and in working over the soil. Most abundant among soil animals are the protozoa. Their activity appears to be correlated with that of bacteria in the transformation of soil nitrates. Two species of *Amoeba*, several flagellates, and ciliates are the predominant members of the protozoan colonies in the soil.

That the quantity of soil microorganisms is exceedingly great is shown by the following figures, in numbers of organisms per gram of soil, obtained at Rothamsted, England.

Bacteria.......	35,000,000	Amoebae.........	200,000
Algae.........	100,000	Flagellates........	600,000

Darwin called attention to the influence exercised by the earthworm in loosening the soil, aerating it, and changing the chemical constitution through digestive processes. Insects, especially ants, and larger burrowing animals contribute their part, for better or for worse, in remaking the soil and thus influencing the life of plants.

Fertility. If a soil contains certain necessary minerals, the requisite amount of organic matter, a suitable subterranean fauna and flora, and is of good texture, it will be fertile ground for most plants; but it may be far better ground for one kind of plant than for another. A soil cannot be said to be fertile except in relation to the particular crop which is to be grown upon it.

Many a pioneer settler, particularly on tropical lands, has been misled into believing that a soil must be fertile because it supports a luxuriant natural vegetation. Agricultural plants have, on the whole, a greater mineral content, and therefore make a greater demand on the soil for salts, than do wild plants. Consequently, a dense natural vegetation is no indication that the land when cleared will be fit for agriculture.

Many problems in tropical agriculture show that the type of crop, and not necessarily soil fertility, often determines yield; thus, grapefruit grafted on sour-orange stock does poorly on certain lean clay soils, but when grafted on rough lemon stock does well on the same soil because the later stock is able to get salts in sufficient quantity to carry on.

Whether a crop is annual or perennial also often determines its success on a soil. Growth of annuals does not indicate that a soil is good or bad for perennials, because annuals need all their salts quickly, whereas perennials have time to accumulate needed salts.

Hoagland has shown that, in order for a plant to take up salts against a concentration gradient, it must have oxygen. Water-logged soil is, therefore, bad not only because of lack of oxygen for respiration, but also because of interference with the intake of salts. The tropical farmer or planter may add fertilizer to a water-logged soil and wonder why no results are obtained, and then be surprised to have a big yield of coconuts on similar soil without fertilizing in a dry year.

There is also the question of seed production vs. vegetative growth. Tonca-bean trees grow well on rich soil but bear poorly, therefore lean soil is better for this plant from an economic point of view.

Man. As the world's population grows, the part played by man in influencing plant life becomes increasingly greater. In old and densely populated countries, as in central Europe, man determines almost wholly what shall grow and what shall not grow. In such regions, the influence of man on plant life is in large measure a beneficial one. Laws, often centuries old, protect plants of economic value and preserve soil fertility. In newly settled countries the situation is unfortunately quite the reverse. The pioneer's life is too strenuous a one for him to think of posterity.

Some years ago Mt. Mitchell, the highest summit east of the Mississippi, was covered with a magnificent forest. A lumber company was given full right to fell the trees. Those not cut down were crushed. The mountain was left a wasted area where fire would rage and erosion complete the destruction. There was no stopping the devastating foresting of the company, for the contract had been given. Under a more enlightened civilization this could not have happened. The denuding of Mt. Mitchell is a minor chapter in the destruction of lands in the United States; and this country is by no means the only or chief sufferer. China, India, Egypt, and East Africa all have their thousands of square miles of waste land, the result of man's indifference to the future.

Deforestation, grazing, and poor farming are the chief causes of the destruction of land fertility. Wasteful cutting of timber is the first step. Grazing then follows lumbering in bringing about ruin. The Caribbean slopes of northern Venezuela are barren wastes owing first to ruthless cutting of forests and then to destructive grazing. Hordes of goats have roamed these slopes until only a few thorny acacias and cacti remain. Erosion completed the devastation. What is there illustrated on a small scale is the story of vast areas in China and India, countries where famines are of regular occurrence.

Man is not wholly to blame, for Nature is often merciless. In parts of India and China, plant life, when left undisturbed by man, cannot cope with either the disastrous floods of wet seasons or the destructive winds of the dry season. Man has learned much; prudent land management has been the policy of the Chinese people since 2700 B. C., but even they have not learned enough.

When the American forestry service was in its infancy, it met with much opposition from legislators who loudly claimed that the protected land would in one season yield a crop of cabbages of more value than all the timber on it. Herein lay the fallacy, that one season's crop is all that need be thought of. Nature, through the years, adjusts crops to the soil and to the climate. Forests usually occur where precipitation exceeds evaporation. If the reverse is true, grasslands are found; and where evaporation is still greater, desert or scrub vegetation alone survives. The phytogeographic map of a country is very similar to the climatic map based on rainfall, evaporation, and temperature. Man ignores this natural adjustment of crops and strives for one "bumper" crop in a single season; he may produce it, but "year in and year out the yield of the grassland is certain, that of the planted fields, never."

Man is learning; he sprays his trees with insecticides and fungicides; he imports ladybugs to destroy aphids; he irrigates, fertilizes, and rotates his crops; but he is still indifferent to many of the consequences of his short-sighted policies. The great dust storms of the western United States are proof of this indifference.

In spite of the evidence to be had from this country, the people of other countries, still in the pioneer stage, farm as wastefully as did our own pioneers. In the interiors of Central and South American Republics natives fell superb forest trees and leave them to rot in order to obtain virgin soil for cultivation. Where the land is hillside, it readily washes and after one or two seasons is unfit for crops. So the frontier farmer pushes back into the primeval forest, moving his hut as he goes, and fells more monarchs to lay bare another patch of ground for his plantings to support his family. Valuable timber which will require a century to replace is destroyed and the land laid waste to produce what could be supplied for a pittance.

How badly man can err in his handling of land is shown by the draining of extensive swamp areas, which to the uninformed would seem to be a very good thing to do. One of the first effects of drainage is the lowering of the water-table, which may bring about the death of the dominant species and leave to another species the possession of the soil, even when the difference in water level is little more than an inch. Frequently, bog country will yield marketable crops of cranberries and blueberries but, if drained, neither these nor any other economic plant will grow on the fallow soil. Swamps and marshes have their drawbacks but also their virtues. When drained they may leave waste land the surface of which rapidly erodes to be then blown away in dust blizzards disastrous to both man and wild beasts.

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CHAPTER IV

PROTOPLASM

Protoplasm is the living substance of which all organisms are composed (Fig. 8). It looks much the same wherever found, yet obviously it cannot be identical in all organisms; otherwise an acorn would not grow into an oak and a frog's egg into a frog. Certain fundamental differences must exist. This is not only true of the protoplasm which gives rise to individuals, but also of the protoplasm in the various cells of the same organism. Cells in different tissues carry on distinct functions; their protoplasm must, therefore, be to some extent dissimilar. A search for the fundamental differences which exist in protoplasm reveals nothing significant. Chemical analyses show a striking uniformity in composition; what variations are found do not appear to be of fundamental importance.

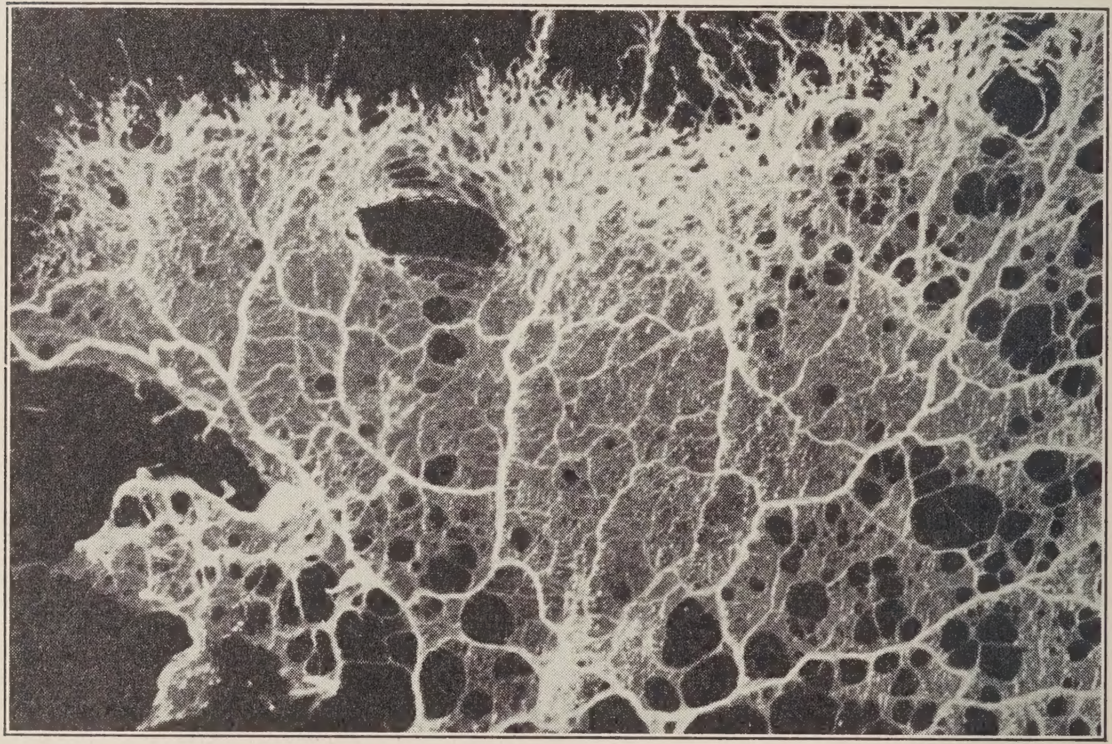

FIG. 8. Slime-mold protoplasm.

Chemical analyses of protoplasm lack the value they would seem to have because, once protoplasm is subjected to drastic laboratory treatment, it is greatly changed. Reagents, heat, or any of the necessary procedures of routine chemical analysis, make of protoplasm a substance quite other than that which it was when alive. The final result may, therefore, give only a superficial idea of the constitution of living matter. However, some of the findings are reasonably significant. The proportion of water present in protoplasm has been frequently determined with moderate accuracy; so also the kinds of elements. In a general way, it is known how much of the solid matter in protoplasm is protein, how much is fat, carbohydrate, and salt. Although this little is all that can be said, it is helpful, and paves the way for more exact work in the future.

Chemical analyses of the plant as a whole suggest the possible constitution of protoplasm, for whereas much of a plant is nonliving matter, it has all been produced by protoplasm.

Water is the "universal solvent" in the living world and may constitute as much as 94 per cent of plant tissue, as in lettuce leaves, or as little as 12 per cent, as in the seeds of lupine. Dissolved in the water are salts of considerable variety. Carbohydrates, too, are abundant, and far more so in plants than in animals; thus, certain seeds, such as wheat and rye, have as high as 68 per cent of sugar, dextrin, and starch. To these carbohydrates is to be added cellulose, the most general constituent of the framework of plants. The nitrogen-containing compounds of plants are proteins, polypeptides, and amino acids.

Organic acids are numerous, among them being oxalic, malic, citric, and tartaric. Members of the fatty acid series, acetic, propionic, and butyric acids, also occur. Fats, oils, and fat-like substances are common storage products in plants, the oils of seeds being examples. The ethereal oils characterize certain plants; among them are oil of turpentine, camphor, and the sweet-scented aromatic compounds. Closely related to the ethereal oils are resins and latex. Alkaloids, such as cocaine and morphine, and glucosides, such as smilacin and digitin, are less common. Pigments are abundant and important plant constituents. Certain of the foregoing compounds found in plants are secretion products rather than constituents of living matter, but the major groups are all represented in the heterogeneous mixture called protoplasm, and in nearly the same proportion.

Metabolically active protoplasm is about three-fourths water and one-fourth solid matter, although this ratio may vary considerably; thus, the streaming protoplasm of a slime mold contains far more water than the hard, brittle, and comparatively dry protoplasm of its own sclerotium. Of the solid matter in protoplasm, one-half is protein and the remainder salts, sugars, and fats. Among these ingredients, the proteins appear to be fundamental.

The following analysis of protoplasm by Kiesel is of the dehydrated plasmodium of the slime mold, *Reticularia.*

	Per Cent		Per Cent
Protein (mostly albumin)	28	Lecithin	5
Nucleic acids	4	Cholesterin	1
Other nitrogen-containing compounds	12	Carbohydrates	23
Fat	18	Unknown	9

Concerning this analysis, many unanswerable questions can be asked: Is this mixture of chemicals really protoplasm? Are the carbohydrates *in* the protoplasm or *of* it? Are all the fats, proteins, etc., just extraneous substances, nutrients, and the 9 per cent unknown matter the protoplasm? An approximation of the truth is all that can be hoped for in such an analysis.

More of significance can be said concerning the physical properties of protoplasm than has been said about its chemical constitution. The laws of classical physics and chemistry, based on pure liquids and true solutions, are applicable to protoplasm only in a general way. They do not always hold strictly, for protoplasm exhibits the same anomalous behavior and possesses the same unique properties which characterize the colloidal state of matter. It is, therefore, in colloidal physics that many of the properties of living matter find elucidation.

Protoplasm is an intimate association of three distinct types of systems, a true solution, an emulsion, and a jelly. The salts and sugars in protoplasm are in true solution. The fats form an emulsion (Fig. 9). The proteins exist as a jelly, which may be either very firm or fluid enough to flow freely.

The foregoing facts may be illustrated by reference to milk. Viewed through the microscope, milk is an emulsion of liquid droplets of butterfat dispersed in an aqueous medium which contains salts and sugars in true solution. When milk coagulates to form clabber or cheese, none of the constituents so far mentioned, the butterfat, the salts, or sugars, plays any part in forming the coagulum; this is accomplished solely by the proteins, primarily the casein of the milk.

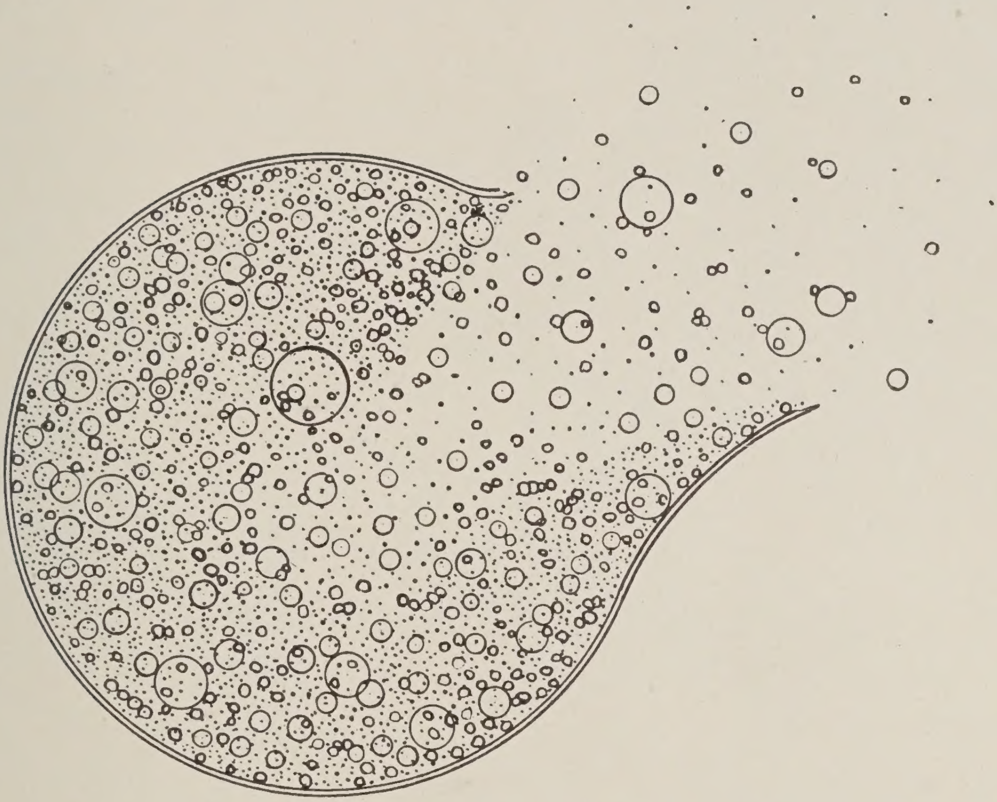

FIG. 9. The coarse protoplasmic emulsion: a torn *Fucus* egg. Compare with Fig. 3.

Physical methods of investigating protoplasm are numerous, involving a variety of optical and mechanical devices. *Dark field* (page 17 and Fig. 6) has been applied with interesting results. *Microdissection* or *micrurgy* (Figs. 10, 11) has revealed much pertaining to the viscosity, elasticity, and structure of protoplasm. With the aid of exceedingly fine and mechanically controlled glass needles held in a micromanipulator (Fig. 10) the cell can be torn apart (Fig. 12), bits cut off, and regeneration powers studied; the nucleus, chloroplast, vacuole, and even the chromosomes may be isolated and dissected. Instead of needles, micropipettes may be used, and salts or dyes injected into protoplasm. Cell inclusions, such as the micronucleus of a protozoan, may be removed with little injury to the cell.

Another very helpful aid in protoplasmic study has been the recently perfected method of culturing slime molds. The cultures may be kept growing on oatmeal indefinitely, thus supplying ample protoplasm for study at all times of the year. The growth may be extraordinarily prolific (Fig. 13). This technique is, however, merely a means for obtaining material for protoplasm studies and not a method of investigation.

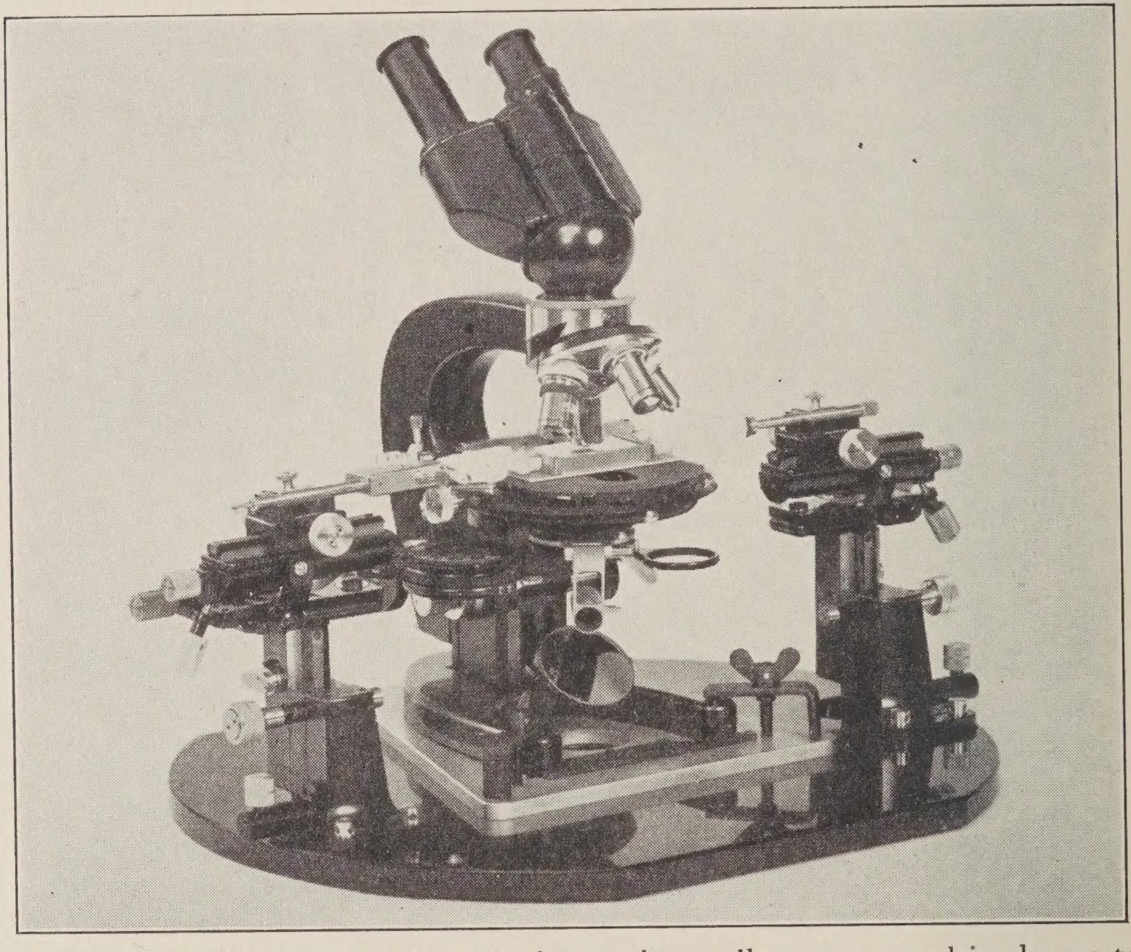

FIG. 10. A Zeiss-Peterfi micromanipulator; the needles are encased in sleeves to prevent contamination. See Fig. 11. (Photograph, Carl Zeiss, Jena.)

In studying any one type of protoplasmic material, the results obviously apply primarily to that material; yet they may apply in large measure to other kinds of material, for many properties are common to all types of protoplasm. Thus, all protoplasm is elastic, weakly so when it is thin, prominently so when it is thick (Fig. 14). All protoplasm is glutinous (sticky), again more or less so at different times. All protoplasm coagulates at death, exhibits pronounced changes in viscosity, and takes up water readily.

Water is imbibed by protoplasm (page 70) as in the case of any gel or sponge, and when the maximum water-holding power of the living or non-living jelly is reached no more water can be taken up. Protoplasm is not freely miscible in water; it is perfused with water, bathed by it, but not dissolved in it. These facts are fundamental in that they give an insight into the structure of living material.

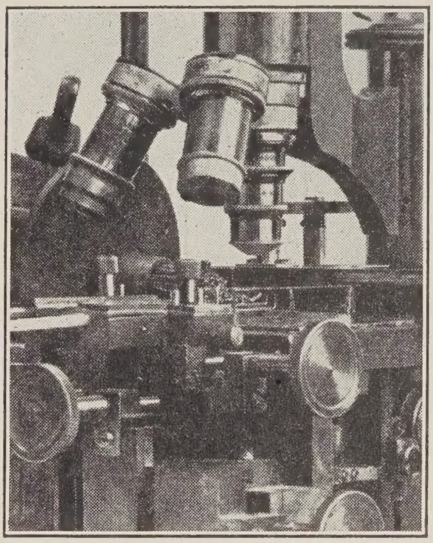

FIG. 11. Two glass micro-needles and a magnetic needle in a moist chamber.

The foregoing properties of protoplasm are expressions of an important feature of its structure, namely, its continuity. The structural units cling together and form a continuous framework, which gives to protoplasm its elastic and rigid qualities.

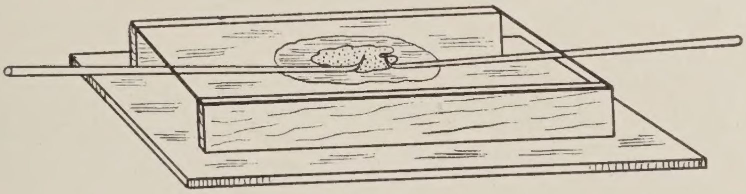

FIG. 12. Protoplasm in the process of microdissection.

The sclerotium, the dry and resting protoplasm, of a slime mold is brittle, whereas the fluid endoplasm of metabolically active cells is but feebly elastic. Jellies, in general, are semi-rigid or semi-fluid systems which flow when thin and are firm and highly elastic when thick.

The structural units of the framework of protein and protoplasmic jellies are either molecules of mammoth size, or molecular aggregates, i.e., micelles. In either case the units are linear, very probably slender, crystalline fibers. With such units it is possible to construct the framework of a system which, with but

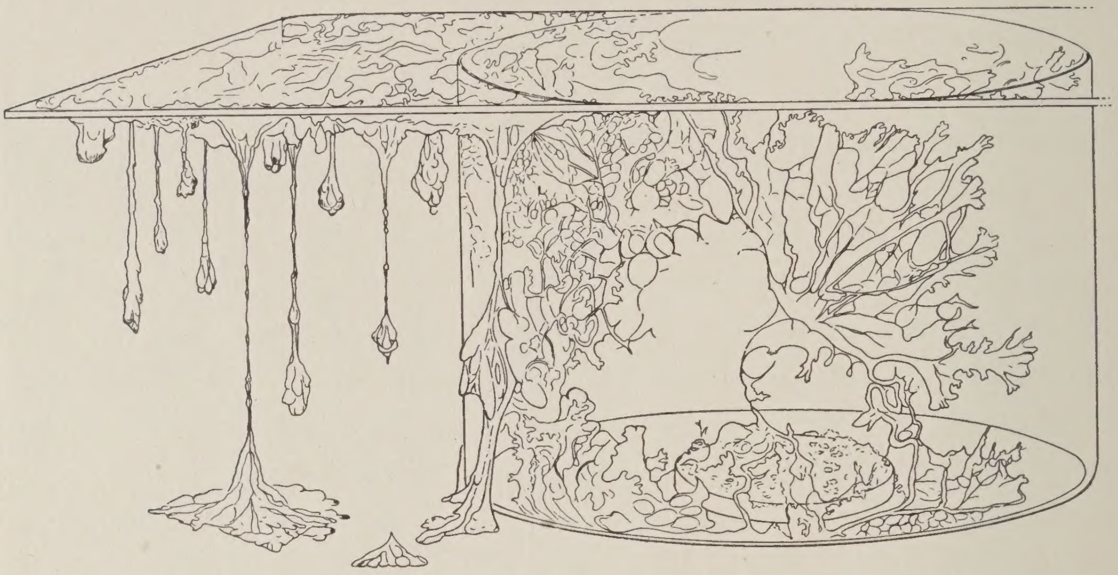

FIG. 13. Protoplasm; a culture of the slime mold, *Physarum polycephalum*. Note hanging lobes of highly viscous protoplasm.

1 or 2 per cent of solid matter, will hold 98 or 99 per cent of water and still possess the firmness of a "trembling" gel. This is remarkably well illustrated in the jellyfish, which is 96 per cent water; yet when stranded it will support the weight of a

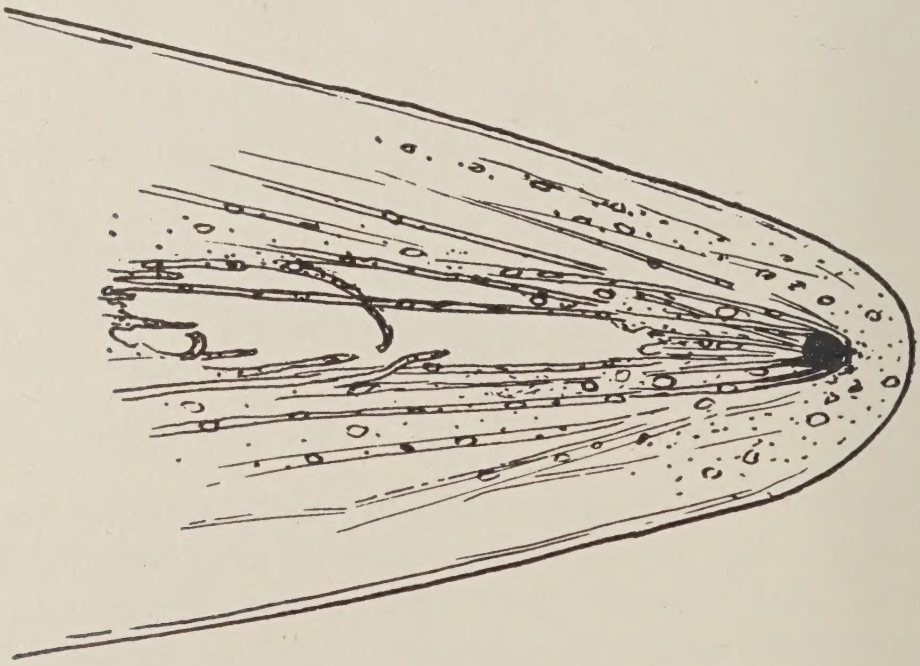

FIG. 14. Torn protoplasm showing fibrous structure.

man. If allowed to remain in the sun for a few hours, the jellyfish will become a thin and barely visible film.

Among the vital activities of protoplasm is its movement. In a slime mold and in *Amoeba*, protoplasmic movement takes two forms, the amoeboid progress of the cell as a whole and the flowing or *streaming* of the protoplasm within the cell body. Protoplasmic streaming is especially characteristic of certain types of cells, but probably takes place to a greater or less degree at different times in all cells. It was formerly looked upon by some physiologists as a pathological condition, in contrast to the view, now generally held, that it is a normal and healthy activity.

The energy responsible for protoplasmic flow has been the subject of much lively and stimulating speculation. Among early suggestions was one which attributed protoplasmic streaming to changes in surface tension, the *sine qua non* of so many cellular activities. There was also the suggestion that the one-way "shuttle" type of flow, where movement is first in one direction and then in the other, as in coenocytes, may be due to hydration at one end and dehydration at the other end; but, as the protoplasm flows equally well in both directions, even when fully submerged, dehydration cannot be primarily responsible.

As colloidal chemistry developed, it became apparent that many cellular activities had their counterparts in colloidal systems. There thus arose the suggestion that protoplasmic streaming is a cataphoretic migration of particles or the electroendosmotic flow of an aqueous medium (page 19); if either, it is the latter, for in streaming protoplasm the entire mass of material moves and not just the suspended particles. In spite of some attempts to prove that streaming protoplasm is associated with electric potentials, there has been no convincing evidence that the potentials measured are real, in the sense of innate to the protoplasm; or, if real, that they are the cause rather than the result of the streaming.

Should the source of energy responsible for protoplasmic streaming be found, there would still remain the question why the direction of flow reverses, as in the shuttle type of streaming.

The protoplasm of slime molds flows but one way at a time. Were a potential responsible, there would have to be a reversal of its polarity every 40 or 50 seconds. The problem thus takes on a twofold aspect—why the streaming, and why the reversal?

The reversal in the direction of protoplasmic flow in slime mold plasmodia is rhythmic. The average of many time records gives 45 seconds for each period of outward flow and 40 seconds for each return period. The average maximum rate of flow is 0.07 mm per second.

When the moving picture of a plasmodium is taken at one-hundredth of the normal speed and subsequently projected at the customary rate, the entire plasmodium is seen to go through rhythmical contraction and expansion similar to the pulsations of the heart. At diastole and systole the direction of flow reverses. The mechanism of protoplasmic movement in slime molds appears, therefore, to be one of rhythmical contraction and relaxation of the plasmodium as a whole, with periods of 40 and 45 seconds for each pulsation. The plasmodium contracts with outgoing protoplasm and expands with incoming protoplasm. This swelling and shrinking is not the result but the cause of streaming; that is to say, contraction is not due to the exit of fluid, and expansion to its entrance, but, as in the case of the heart, the exit and entrance of fluid are due to the contraction and expansion of the living substance.

The beat of the heart is controlled by a sympathetic nervous system. There is no nervous system in the sense of nerve centers and nerve fibers in slime molds, but these are not necessary in order to have nervous response and control.

Some modern workers are inclined to strip protoplasm of all the attributes of higher organisms, forgetting that many of these attributes exist because they are properties of protoplasm. There is a tendency to regard the protoplasm of primitive forms of life as less intricate, less responsive, if not "less living" than that of more highly developed forms. If it is granted that protoplasm is alive, it must be admitted that it exhibits irritability and nervous response. It is strange that the remarks of Hales, "vegetables (which are inanimate)", should in a vague way persist in the minds of some.

There is possibly a phylogenetic relationship between the rhythmical pulsations responsible for protoplasmic streaming in myxomycetes and the rhythmical expansion and contraction of sympathetically controlled muscle tissue.

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CHAPTER V

THE PLANT CELL

Protoplasm is the living substance. Ordinarily, it occurs as organized individual droplets of microscopic size which, in plants, are usually enclosed in walls of cellulose. Such units are *cells* (Fig. 15).

All organisms are built of cells; protista are *unicellular*; higher organisms are *multicellular*. Cells combine to form *tissues*, and tissues form the plant or animal as a whole.

The term "cell" was coined by Robert Hooke in 1665. With his primitive microscope, he saw tiny cavities in cork which, being empty, he called "cells." No protoplasm was there, for cork is dead tissue, so he could not have had in mind the essential material of cells. In order to distinguish more clearly between the casing of cellulose, which Hooke saw and called cell, and the contents of protoplasm, the latter, considered as a unit, is termed a *protoplast*. All animal cells are protoplasts, for they lack heavy walls. Although the term protoplast is convenient, cell has come to mean the same thing. Were the botanist to adhere rigidly to cell for the casing of cellulose, and always say protoplast in reference to the enclosed protoplasm, he would find himself in frequent conflict with the zoologist, for whom the protoplast is the cell.

Every protoplast is enclosed in a delicate membrane, which consists of protoplasm, more or less differentiated from the inner material. A cell, therefore, may be defined as a mass of protoplasm surrounded by a membrane. The protoplasmic membrane is the surface layer of a protoplast, whether animal or plant. In the cells of higher plants the protoplasmic membrane not only encloses the protoplast but also lines the inner surface of the cellulose wall.

Plant cells exhibit great variety in external form and internal organization. The typical mature plant cell has a single, large central vacuole, and a thin peripheral layer of protoplasm, with imbedded nucleus and plastids, the whole surrounded by a delicate membrane and a wall of cellulose (Fig. 15).

Vacuoles are characteristic of cells. It is doubtful whether any cell, plant or animal, is wholly lacking in them. Plant vacuoles are sacs containing salts and organic matter in aqueous solution. The vacuole possesses a membrane known as the *tonoplast.* It and the outer protoplasmic membrane are very important physiological structures, for they determine the osmotic and selective properties of the cell (pages 54, 119).

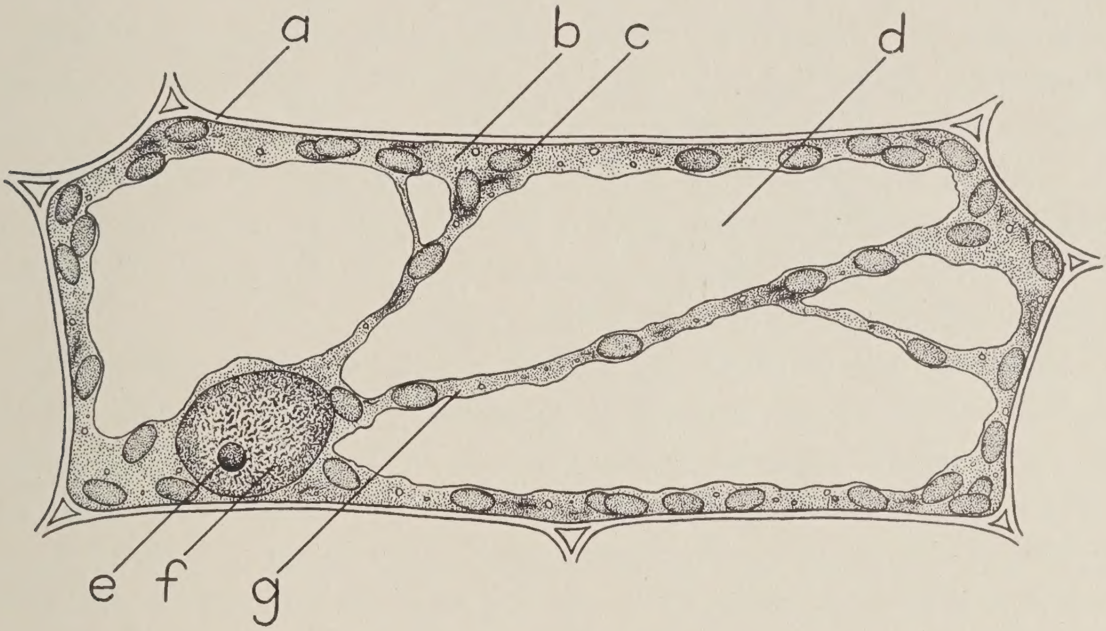

Fig. 15. A plant cell showing (*a*) cellulose wall, (*b*) cytoplasm, (*c*) chloroplasts, (*d*) vacuole, (*e*) nucleolus, (*f*) nucleus, (*g*) protoplasmic strand.

Among cell parts, the *nucleus* holds first place as a regulator of cell activities. There are some exceptions to this, particularly among the lower forms of plant life. Neither bacteria nor blue-green algae possess a true nucleus, but they do presumably possess nuclear material, arranged as a *central body* in the blue-green algae. Among higher plants there is only one indubitable case of a living cell without a nucleus; it is the mature sieve tube in the phloem of stems. This cell contains living protoplasm, yet has no nucleus. As continued life without a nucleus is usually impossible, it has been assumed that the nucleus of the small companion cell which adjoins each sieve tube serves for both. The often cited case of mammalian erythrocytes as examples of living cells without nuclei is not a convincing one, as there is no conclusive evidence that these cells are alive when in the blood stream.

Plastids are cell inclusions of various kinds and functions. The most conspicuous among them are the green ones or *chloroplasts* (Fig. 15). Plastids are of great physiological importance, at least in those instances where their function is known. Green plastids are centers of photosynthetic activity, the pigment or *chlorophyll* which they contain functioning as a catalyst or activator of photosynthesis. Certain plastids, known as *leucoplasts*, are colorless. Some, the *chromoplasts*, contain other than green pigments. *Mitochondria* may be regarded as minute plastids, their function being unknown.

Cell inclusions include granules of food, such as the *aleurone grains* of protein; starch grains, the lamellation of which is very characteristic (Fig. 45); and other reserve and waste products, among which are calcium oxalate crystals.

Another cell structure of fundamental importance in physiology is the group of delicate protoplasmic strands or *plasmodesmata* (singular, *plasmodesma*) which establish vital connection between one cell and another through the intervening wall of cellulose (Fig. 16). Plasmodesmata have been the subject of much controversy, with the majority of evidence first on one side and then on the other. The difficulty lies in distinguishing with certainty whether an observed line is a protoplasmic strand, a ridge, a groove, or a pit in the wall. Němec has long been of the opinion that these lines passing from one cell to another through the wall are of protoplasmic material. A vital connection between cells is to be anticipated, though not necessary, in order to account for the transmission of stimuli, such as takes place in the sensitive plant, *Mimosa* (page 273); but stimuli may be sent through non-living material (page 208). In animals, the question of intercellular connection does not, or need not arise, for their cells are in direct contact with each other, protoplasm touching protoplasm.

The interrelationships between form and function and between cell and environment are matters which, when given due consideration, show the cell capable of far greater adjustment and adaptation than is usually accorded it as a unit in the multicellular organism. When cells are first formed, in regions known as *meristems*, they all look very much alike; but differentiation soon sets in and the cell takes on the structure and function of a definite tissue cell. The form of a cell usually suits the function; thus, epidermal cells are locked together, which is made possible by their irregular shape; xylem cells are long tubes adapted to convey water; bast fibers have heavy walls giving them strength; stinging hairs are sharp-pointed cells which penetrate the skin of an animal readily (Fig. 17). The architecture of a cell is a reflection of its activity.

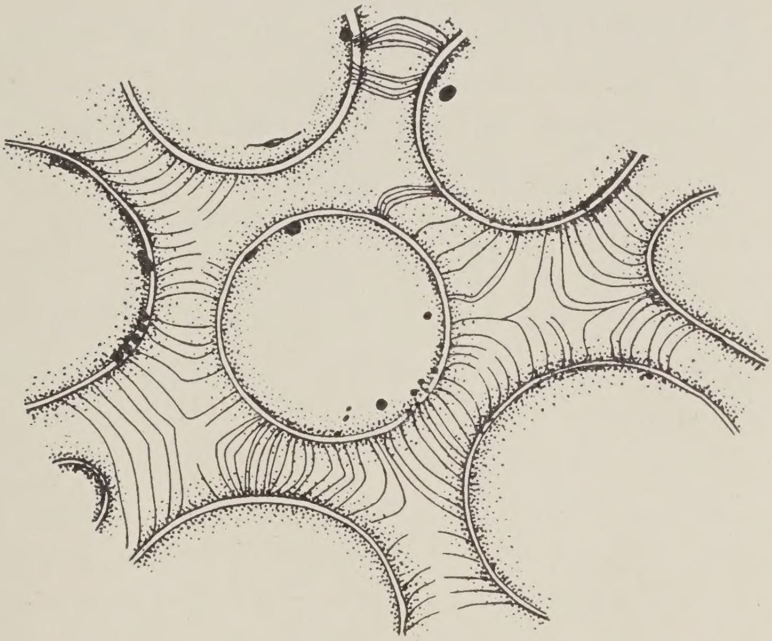

FIG. 16. Plasmodesmata connecting the cells of the persimmon, *Diospyros*. (Drawn from a preparation by C. J. Chamberlain.)

Specialization in the form of cells may be very great. An interesting case of this is the cells of the protonema of the moss, *Schistostega osmundacea* (Fig. 88).

The greatest specialization in cells is attained in protista, such one-celled plants and animals as bacteria, blue-green algae, diatoms, and protozoa, each one of which is not only a cell but an organism. Protista are often far more complicated than tissue cells.

The concept "cell" may, therefore, mean anything from a

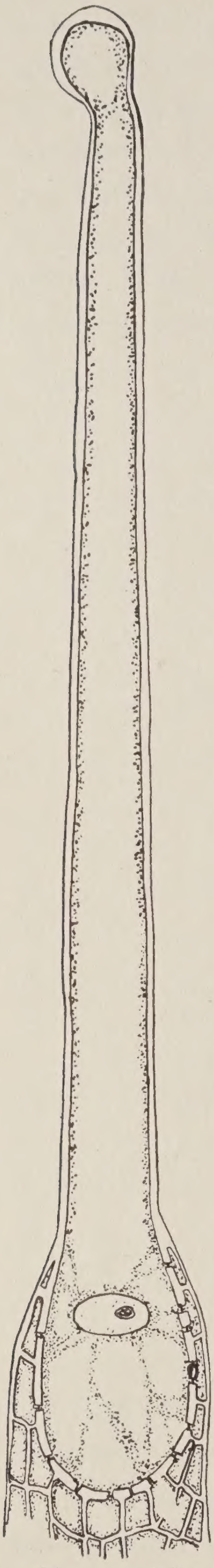

FIG. 17. The specialized cell of the stinging-nettle fashioned after the manner of a hypodermic needle.

relatively simple, undifferentiated protoplast which is part of a larger whole, to a highly organized one-celled plant or animal, including the large coenocytic and multinucleate plasmodia of slime molds which may be several square inches in area (Fig. 8).

Again let it be stated that in emphasizing the complexity of protista injustice must not be done to the apparently simple tissue cell which has hidden in its protoplasm potentialities that may equal those of a one-celled organism; thus, a pith cell may, on occasion, develop into any kind of tissue cell to be found in the plant.

The form and function of cells are determined in part by the protoplasm of which they are made and in part by environmental influences. No living cell can be dissociated from its environment. Tissue cells function as separate individuals to a limited extent only; for example, the cell in a leaf is a microscopic laboratory where the synthesis or building up of food takes place, but the process does not go on independently of other cells; water, carbon dioxide, and other substances out of which organic compounds are formed, must be supplied by cells elsewhere in the plant. The situation is very much the same as in a factory where each department operates more or less independently but would not function long were it not receiving material from and delivering it to other departments. In the same way no tissue cell is wholly independent of its environment. This is equally true of the relationship between a cell and the fluid which bathes it, whether the body fluid or the solutions of the sea, of inland water, or the soil.

The Root Hairs. All cells play a part in the life of the organism as a whole, but certain ones are singled out for very special duties. Such an honor is accorded to the root hair (Fig. 18). Nearly all water and salts which enter the plant from the soil do so through root hairs. Water may enter any living superficial cell if it is not heavily cutinized or made otherwise poorly pervious. In roots, this amount is slight compared with the supply which enters by way of the root hairs.

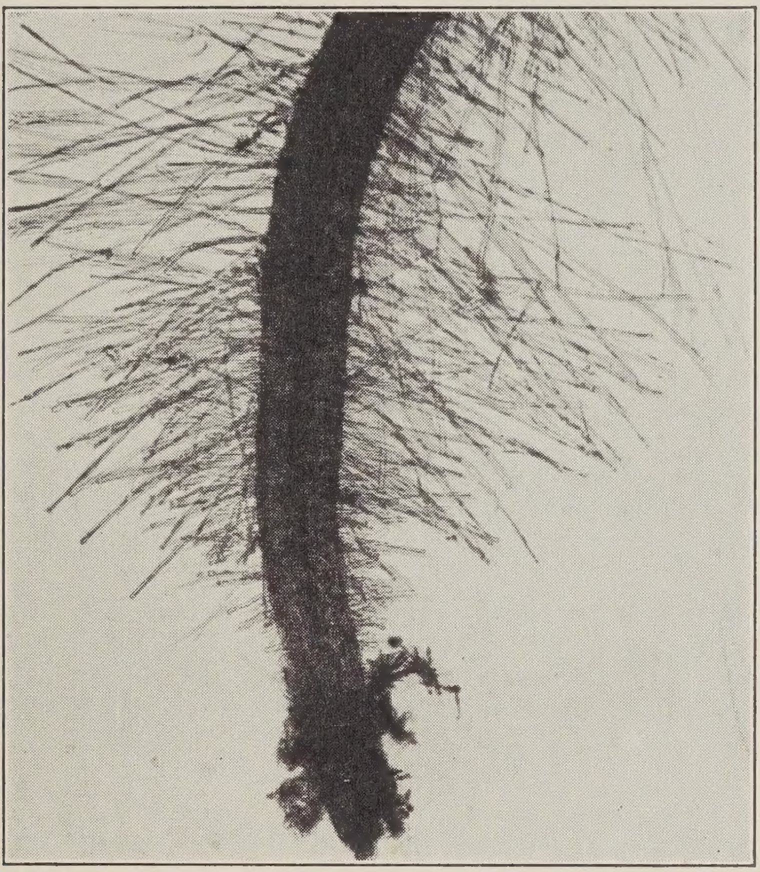

Fig. 18. Root tip of *Nicotiana* with root hairs. (Photograph, Philip R. White.)

The root hair is an epidermal cell which has become greatly elongated. It is always a thin-walled cell. The wall appears to be primarily of pectic material (calcium pectate) rather than of cellulose, as is true of most cell walls. The vacuole is large, and the protoplasm is usually actively streaming.

Nearly all young roots possess hairs near their tips. Owing to the shape and abundance of root hairs (Fig. 19) the absorbing surface of roots is thereby greatly increased (Fig. 20). Many plants which live in water, such as the water lily, do not have root hairs. Whereas this is generally true of aquatic plants, it is not an infallible rule, for some aquatics, such as *Trianaea*, develop abundant root hairs.

The production of root hairs is to a great extent determined by surrounding conditions. The roots of corn develop a thick covering of root hairs near their tips when in soil, but have few if any hairs when in water solutions, whereas the waterweed, *Elodea,* forms no root hairs when its roots are free in water, but, as soon as they enter the soil, hairs are produced. Extreme dryness as well as excessive moisture retards root-hair development; thus, some air roots which are normally without hairs develop them when they come into contact with the earth or a moist wall.

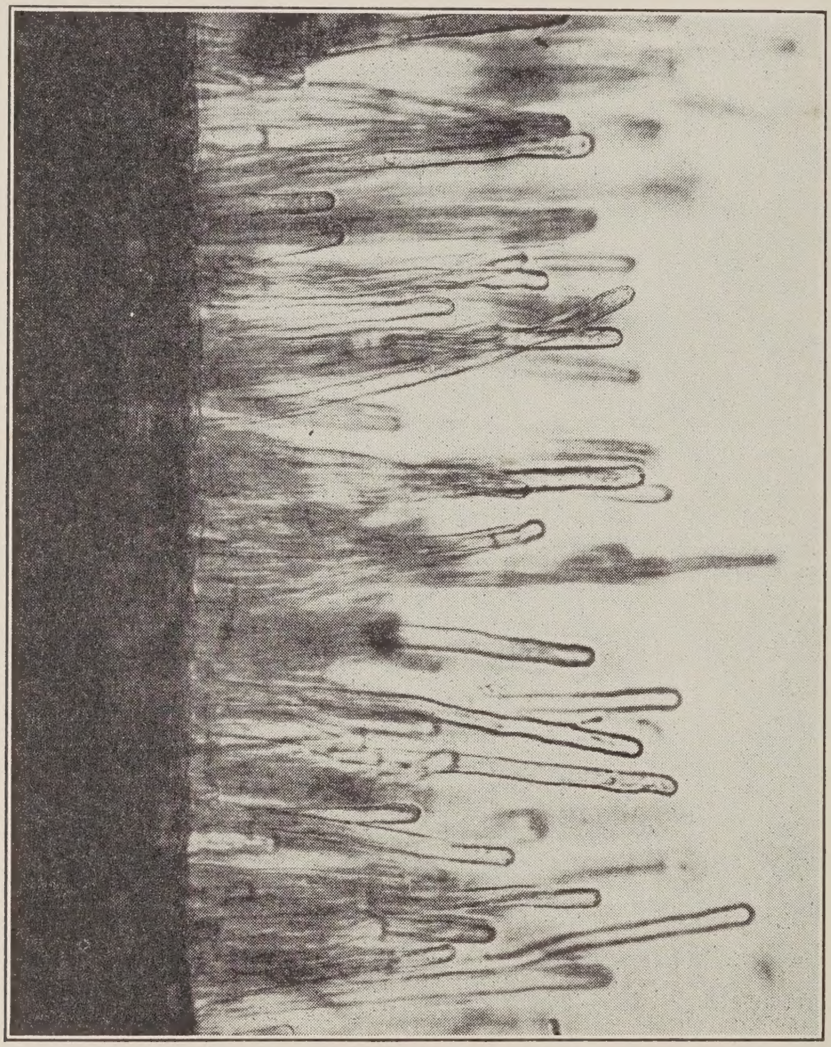

FIG. 19. Root hairs of *Solanum.* (Photograph, Philip R. White.)

No structure is more important to the well-being of the plant than the root hair. It is the gateway of the vast amount of water used in photosynthesis and all metabolic processes and given off in transpiration. How the root hair functions as an osmotic system forms the subject of an entire chapter to follow (Chapter VI).

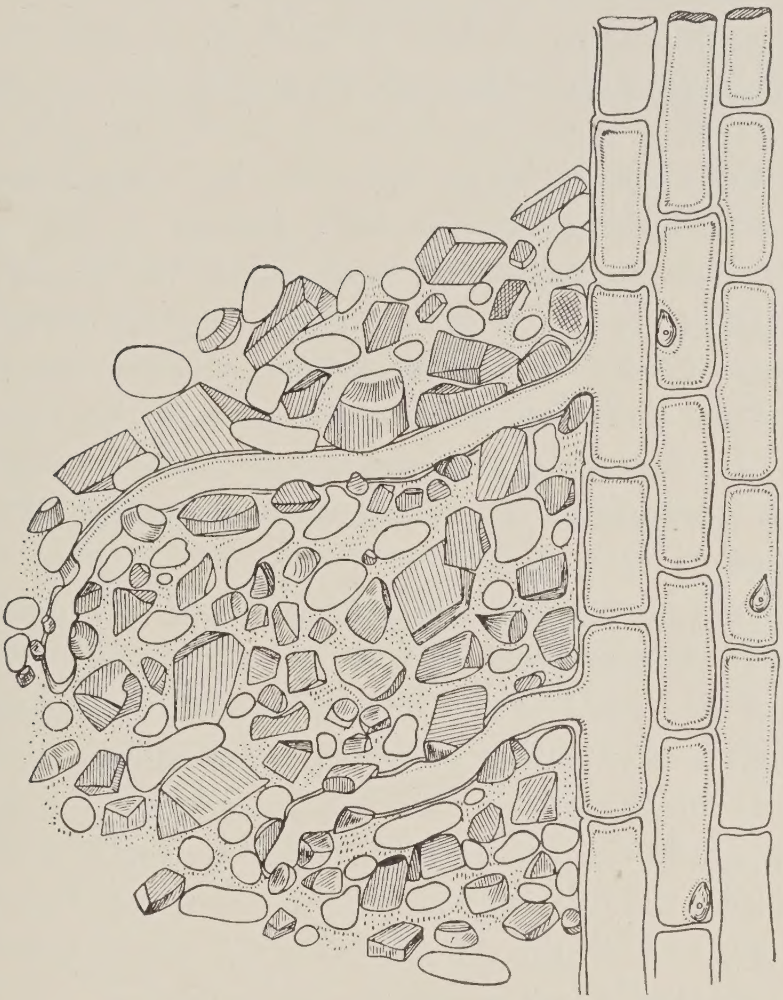

FIG. 20. The root hair in contact with soil particles, soil air, and soil water.

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CHAPTER VI

OSMOSIS AND THE ENTRANCE OF WATER

Among the physical forces operative in a living plant certain ones are outstanding because they appear to be universally present, or because numerous functions have been assigned to them, or simply because they have been very extensively studied. The pressure or *turgor* resulting from *osmosis* qualifies in all these respects. It stands preeminent among the properties of plant cells. Its discovery and subsequent study constitute one of the most interesting series of events in botanical and chemical annals (page 5).

The delicate protoplasmic membrane which surrounds all protoplasts is readily permeable to water, but not to salts, sugars, and many other materials. Cells are normally bathed in solutions the salt concentration of which is less than that within the cell. Under such conditions more water enters a cell than leaves it, until an equilibrium is reached. This results in a hydrostatic pressure within the cell. The proportion of excess water diffusing into a cell varies with the differences in the concentrations of the dissolved substances in the surrounding solution and in the cell sap. The movement of water under these conditions is *osmosis*; the pressure produced is *turgor*; and the pressure which would have to be applied to prevent the entrance of water is the *osmotic pressure* of the cell sap.

The conditions which make an osmotic system of the living plant cell are not unique to it; they may be readily duplicated artificially. If a solution of sugar is put into a sac of parchment paper and the whole placed in water, water will enter, and in so doing produce a hydrostatic pressure within the sac. The parchment paper, like the protoplasmic membrane, is readily permeable to water but not to dissolved substances. The solution within the sac corresponds to the cell sap which is more concentrated than the surrounding solution. These are the conditions necessary for osmosis (Fig. 21).

That water does enter living cells osmotically so as to produce a pressure is readily demonstrated, and conveniently so by using a carrot or beet as the osmotic system. A cork borer is pressed into the root at the top and a hole, two or three inches deep, is made. This is filled with concentrated sugar solution or a thick syrup, and a glass tube attached with a cork and sealed with wax. The root is then immersed in water. In time, the solution from the beet will rise five to ten feet in the tube.

Membranes which permit water to pass through, but keep out dissolved substances, are said to be *semi-permeable.* No natural membranes, nor most artificial ones, are wholly semi-permeable, for they allow a certain amount of the solutes to pass through. They are, therefore, better spoken of as *selectively* or *differentially permeable.*

If water enters a cell or artificial sac having a selectively permeable membrane which encloses a solution of higher concentration than the surrounding solution, then water should leave the cell if the concentrations of the solutions within and without are reversed. The entrance of osmotic water is known as *endosmosis* and its exit as *exosmosis.* Excessive exosmosis of water brings about a reduction in turgor which, if carried far enough in a living cell, will result in the withdrawal of the protoplasmic membrane from the cellulose wall. This phenomenon is known as *plasmolysis* (Fig. 22). Plasmolyzed protoplasts may assume a variety of forms (Fig. 23).

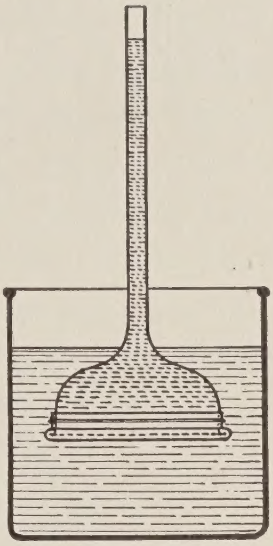


FIG. 21. An osmotic system: a semi-permeable membrane tied over a thistle tube containing a sugar solution. Water is without.

The cause of the excess movement of a solvent in one direction through an osmotic membrane is best interpreted in terms of difference in vapor pressures, or escaping tendencies, of water and solutions. If two dishes, one containing water and one a solution, are placed under a bell jar, water will evaporate at a greater rate per unit area from the pure-water surface than

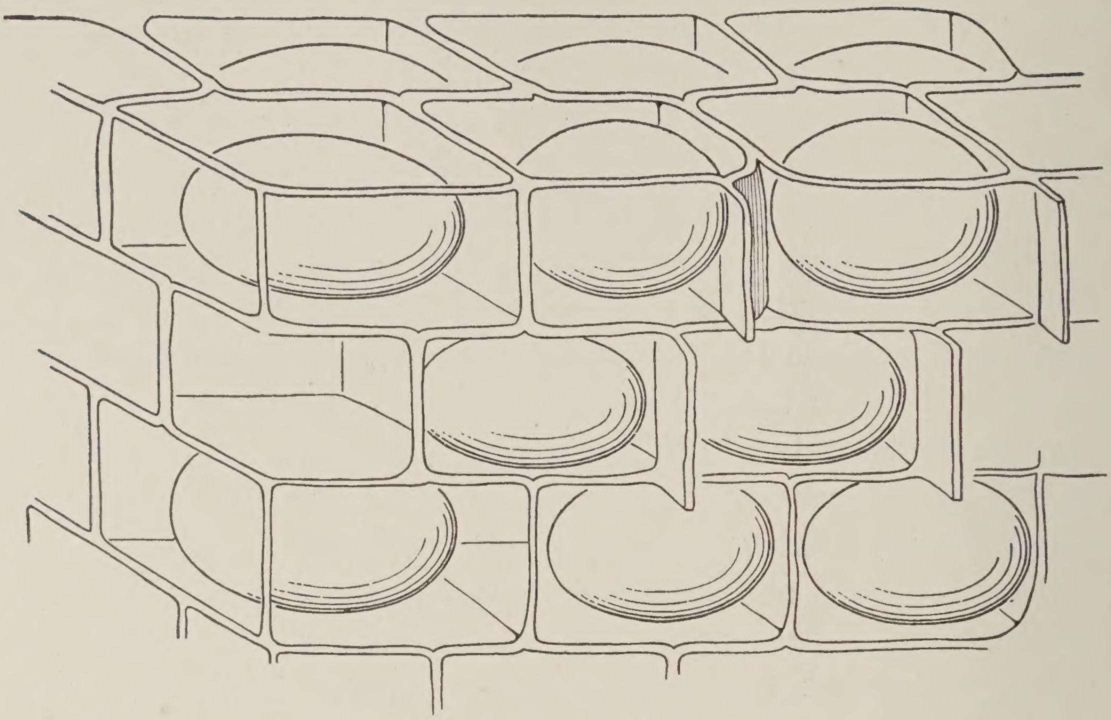


FIG. 22. Plasmolyzed cells in perspective.

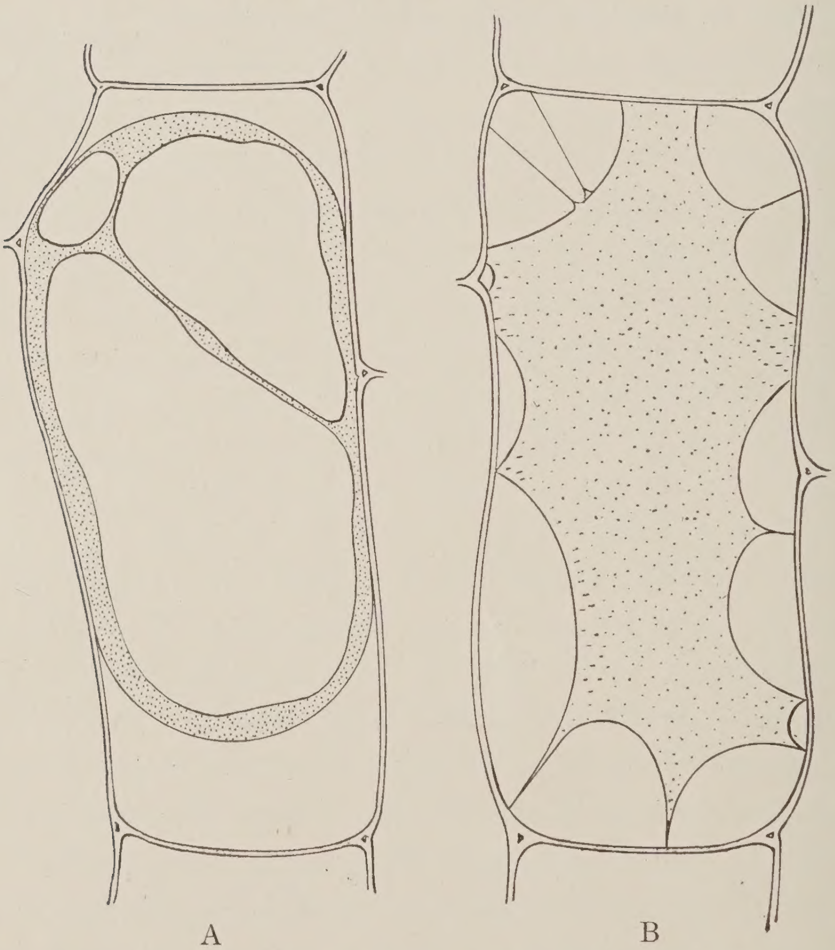


FIG. 23. Plasmolyzed cells: (*a*) convex protoplast; (*b*) concave protoplast with adhesion of the protoplasm to the cell wall.

from the solution surface, because more water molecules per unit area are exposed on the water surface. This means a solution has a lower vapor pressure or escaping tendency than its solvent; in other words, a solution is not in equilibrium with its solvent, at the same temperature and pressure. This being true, water will be condensed on the surface of the solution. In such an experiment, the air takes the place of a semi-permeable membrane. Were the solution confined in a sac, pressure or turgor would result.

A definition of osmosis will depend somewhat on one's point of view. The phenomenon was first observed by a physiologist. As a result, biologists in general have always associated osmosis with a selectively permeable membrane such as surrounds living cells (see also page 120). The physical chemist, on the other hand, views the problem thermodynamically and regards the condition involving a membrane as merely a special case. For the physiologist, osmosis is the excess diffusion of a solvent from a solution of greater concentration of solvent to one of lesser concentration of solvent through a membrane permeable to the solvent but not to the solute. As the experiment with the two dishes under a bell jar cited above illustrates that no morphological membrane is necessary, osmosis may be defined as the process by which one liquid increases in volume at the expense of another from which it is separated in such a way that flow from one to the other is impossible.

Usually in nature it is a membrane which separates the two solutions of an osmotic system, but any septum will do. In the two dishes under a bell jar, air is the septum. As a result of undue emphasis on the peculiar qualities of natural membranes which permit one kind of molecule to pass through but not another, the membrane has been regarded as the essential feature of osmosis. This is not true. It is the relative escaping tendencies of the solvent of the two solutions which are significant for osmosis. The septum separating the two solutions in an osmotic system may be solid, liquid, or gas and of any thickness. The sole function of the septum is to prevent the two solutions from mixing mechanically, by convection currents, for instance, and so insure molecular movement. The membrane does not *make* osmosis take place. Hence to emphasize it is misleading, as when one says osmosis is diffusion through a membrane. The membrane must, to be sure, possess certain qualities to meet the conditions of the experiment; it must be strong enough to withstand the pressures put on the solutions and yet permit the molecular movement of that constituent the osmotic pressure of which is involved.

Numerous other false ideas have arisen in regard to osmosis. Thus it is said that the water which enters an osmotic sac cannot leave because it is held by the sugar or other particles within; and, as they cannot leave, neither can the water which is bound to them. Such reasoning is wholly fallacious; the problem which it is supposed to solve does not exist, nor does it arise when one reasons thermodynamically. Perhaps the most frequent errors made are the following incorrect definitions of osmosis—"diffusion through a membrane" and "diffusion from a solution of low concentration to one of high concentration." Again the reasoning is faulty; water diffusing through a membrane is not osmosis, nor is there diffusion contrary to the fundamental rule that diffusion is always from a region of higher to one of lower concentration. The error in the latter case comes from observing the diffusion of the solvent, water, and expressing the concentrations in terms of the solute, sugar. Osmosis is but one example of a much more general law, that everything moves from where it is abundant to where it is scarce, in so far as other features of its environment permit. In osmosis the respective abundance or scarcity of water is the important factor, not the abundance or scarcity of solutes. Furthermore, amount of material is of but accessory significance, the energy of movement being all that is important.

If osmosis is simply a special case of a more general law, then, one might ask, why has it been singled out? This is done because striking results follow the separation of water from one of its solutions. Chief among these is the increase in volume of the solution at the expense of the water. This is why osmosis was so named. The chemist finds nothing remarkable about matter moving from a region in which surface energy is great to one where it is less; but osmosis involves other effects than redistribution of matter. It is the change in volume which is so striking and of such tremendous biological importance.

Further confusion has arisen because of the error which lies in thinking of osmotic pressure as the *actual* pressure produced within the cell or sac. *Turgor is the pressure produced; and turgor is not osmotic pressure.* The pressure which results is rarely the maximum which is theoretically possible in an ideal osmotic system, and it is never the theoretical maximum of the original solution, because, with the entrance of water there has been a dilution of the solution. Osmotic pressure must be thought of as an evaluation or rating rather than as an actual pressure. For this reason, *osmotic value*, has been suggested; but, as turgor expresses the actual pressure produced, no confusion results if osmotic pressure is viewed simply as a rating, just as one speaks of the horsepower of an engine to indicate the potential power of the engine, i.e., the power it would produce under ideal conditions. Similarly, osmotic pressure is the capacity of a solution to produce a certain maximum turgor under ideal conditions.

Whether the liquid outside the cell or sac is pure water or a solution is unimportant; it is only necessary that the inner and outer liquids be of different concentrations. That solution which has the higher concentration of solute, and lower concentration of solvent, and therefore the one toward which excess diffusion of water is taking place, is said to be *hypertonic* to the other *hypotonic* one. Two solutions of like concentration and therefore of like osmotic pressure are said to be *isosmotic* or *isotonic* to each other.

Among artificial semi-permeable membranes one has become classical because its semi-permeable properties are more nearly perfect than most other membranes, and because it imitates, in a superficial way, the osmotic properties of cells, their growth and repair. This is a membrane of copper ferrocyanide, and is formed when copper sulphate comes into contact with potassium ferrocyanide. If a crystal of the sulphate is dropped into a dilute solution of the ferrocyanide, a membrane is immediately formed around the crystal (Fig. 24). This precipitation membrane is permeable to water but not to the ions of copper sulphate which it encloses. As a result, water enters the sac and creates a pressure which distends the membrane. The membrane, being inelastic, bursts and establishes a new point of contact between the outer and inner compounds. A new precipitation membrane is formed over the "wound" and the enlarged osmotic sac swells by turgor and bursts again. Thus does the sac grow in size. This phenomenon was first described by Traube, and the membrane is known by his name.

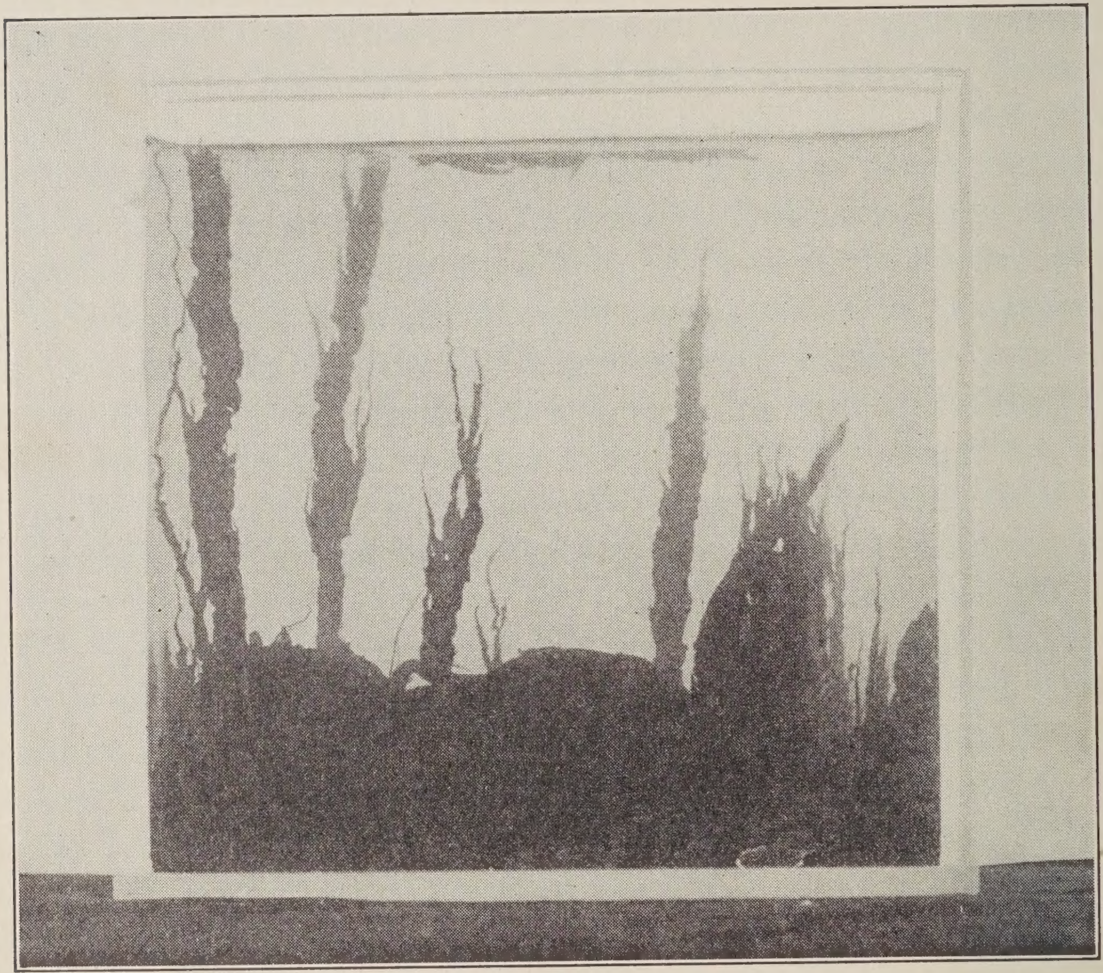


FIG. 24. A "marine garden." Both the "rocks" and the "growing seaweeds" are enlarging osmotic sacs of copper ferrocyanide, with potassium ferrocyanide within and copper sulphate without.

The Traube precipitation membrane has served in very exact determinations of osmotic value. It is a delicate membrane, but when precipitated in a porous clay thimble, by placing copper sulphate in the thimble and bathing the latter in potassium ferrocyanide, it is firmly held and will withstand very high pressures (Fig. 25).

Early work on osmosis demonstrated that the pressure developed depends upon the degree of permeability of the membrane for the solute. Pfeffer found that at the same temperature a solution of sugar developed twice the turgor in a parchment-paper sac as when in an animal bladder, and twenty times as much when enclosed in a copper-ferrocyanide membrane. This experiment adds emphasis to the distinction between turgor, or the actual pressure developed, and osmotic pressure, or the rating of the solution, for in each of the above three experiments the solutions and therefore the osmotic pressures were the same, but the turgor pressures developed differed.

Why some membranes are freely permeable to water but not to solutes is difficult to answer. There are several hypotheses which will be more fully considered in another connection (Chapter XI). Here the question is easily and perhaps rightly answered by the assumption that the membrane is a sieve with pores which let small molecules pass through but not large ones. There are, however, some difficulties with this theory in that big molecules occasionally get through some membranes whereas little ones do not. Wilhelm Ostwald suggested that the electrical properties or sign of the charge on membranes may permit one type of ion, positive or negative, to pass but not the other type; but sugar is not an ion. Possibly, both mechanical (sieve) and electrical (charge) properties are at work.

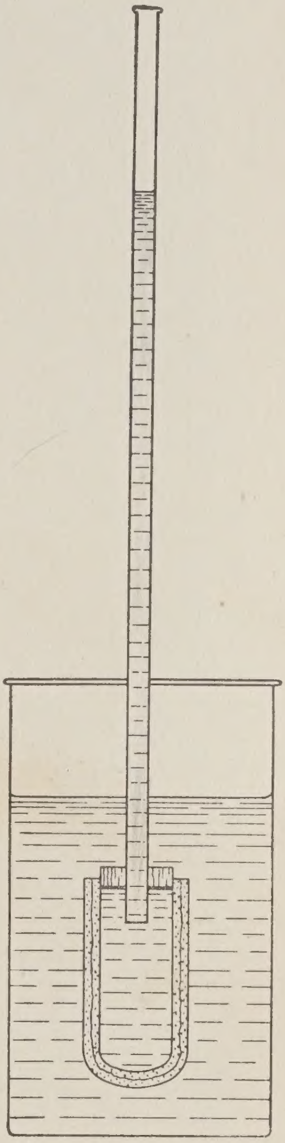
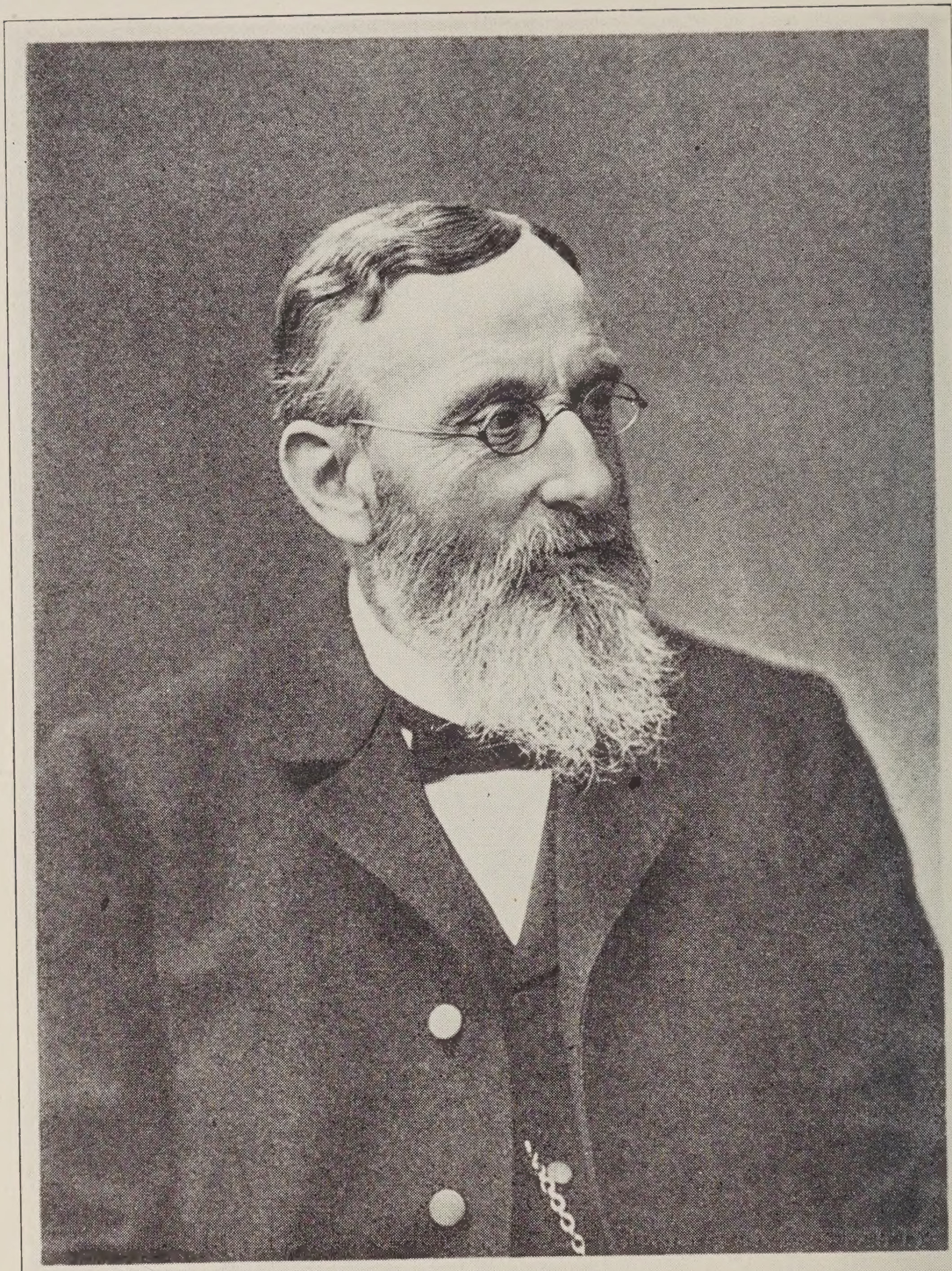


FIG. 25. An osmotic cell of a copper ferrocyanide membrane precipitated in the wall of a clay cup.

Ways of measuring osmotic pressure are several. The *direct* method involves an osmotic system such as that already described. If the osmotic sac is connected by a tube to a manometer or instrument for measuring pressure, the pressure developed may be read directly. It will have a value of 22.4 atmospheres, if *at equilibrium* the solution



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W. Pfeffer

FIG. 26. Wilhelm Pfeffer, 1845-1920. (Photograph, J. Dörfler.)

contains 1 mole of solute in 1 liter of solvent. This pressure will be doubled (44.8 atmospheres) if the salt is a divalent one and fully ionized.

Because technical difficulties make the direct method of measuring osmosis difficult *indirect* methods are usually employed with much greater accuracy.

The *plasmolytic* method is a familiar one commonly employed in plant physiology. Cells are plasmolyzed with known solutions until that one is found which barely does not cause plasmolysis. It is then isosmotic with the cell sap, and, if its osmotic pressure is known, that of the cell sap is also known.

For the physicist, measurements of *vapor pressure, elevation of the boiling point*, and *depression of the freezing point* are the three usual ways of determining the osmotic pressure of solutions. The first depends upon the fact that a solution has a lower vapor pressure than its solvent, and, from this, elevation of the boiling point with increase in osmotic pressure follows. Of the three methods, measurement of the depression of the freezing point is best. Once these values are correlated with osmotic pressure, the latter can be determined by the former.

Dutrochet was the first to observe osmosis, in 1827, and to give an interpretation of it. He explained the bursting of the zoosporangia of algae on the basis of an internal hydrostatic pressure set up by entering water. He experimented further by placing a solution in an animal bladder and putting the whole into water; the bladder burst, and he interpreted the event in the same way as he did the bursting of a zoosporangium.

Fifty years later the plant physiologist, Wilhelm Pfeffer (Fig. 26), made a careful study of osmosis, to which he was led by his studies of plasmolysis. Pfeffer's measurements of osmotic pressure long remained the only exact ones made. Not until the thorough work of the chemists Morse and Frazer in America was Pfeffer's work outdated. Pfeffer perfected the precipitation of the copper ferrocyanide membrane in the walls of porous-clay cups (Fig. 24). The following pressures (in centimeters of mercury) were found by Pfeffer for sugar solutions at constant temperatures (15°C).

Concentration	Pressure
1 per cent	53.2
2 per cent	101.6
4 per cent	208.2

The following values were obtained at different temperatures and constant concentration:

Temperature	Pressure
6.8°	50.5
13.7	52.5
22.0	56.7

Pfeffer was not unaware of the significance of his results, but it remained for van't Hoff (1885) to show, on the basis of the above experiments, that the gas laws are applicable to solutions. As in gases, the osmotic pressure of solutions is proportional to the number of molecules present, temperature and volume remaining constant (Avogadro's law); and, as in gases, osmotic pressure varies directly as the temperature, concentration and volume remaining constant. The following experiment shows this to be true.

A gram-molecular weight or "mole" of a gas (i.e., the molecular weight in grams, e.g., 44 grams of CO_2) occupies 22.4 liters at zero temperature and atmospheric pressure. This is confirmable by experiment. If the pressure developed by a substance in solution is the same as that which it would exert if converted into a gas of the same volume and temperature, then 1 mole of it dissolved in 22.4 liters of water should give an osmotic pressure of 1 atmosphere. As equimolecular concentrations of all substances have the same number of molecules, then 44 grams of carbon dioxide and 342 grams of sucrose will each have the same number of molecules and should, therefore, if the gas laws apply to solutions, yield the same pressures, temperature and volume remaining the same in both instances. The pressure of 1 mole of a gas is 1 atmosphere when it occupies 22.4 liters at 0°C. If the volume of the gas (22.4 liters) is reduced to 1 liter, then the pressure will be 22.4 atmospheres. If all hypotheses are correct, then an equimolecular concentration of sugar in solution (1 mole of sugar) should yield 22.4 atmos-

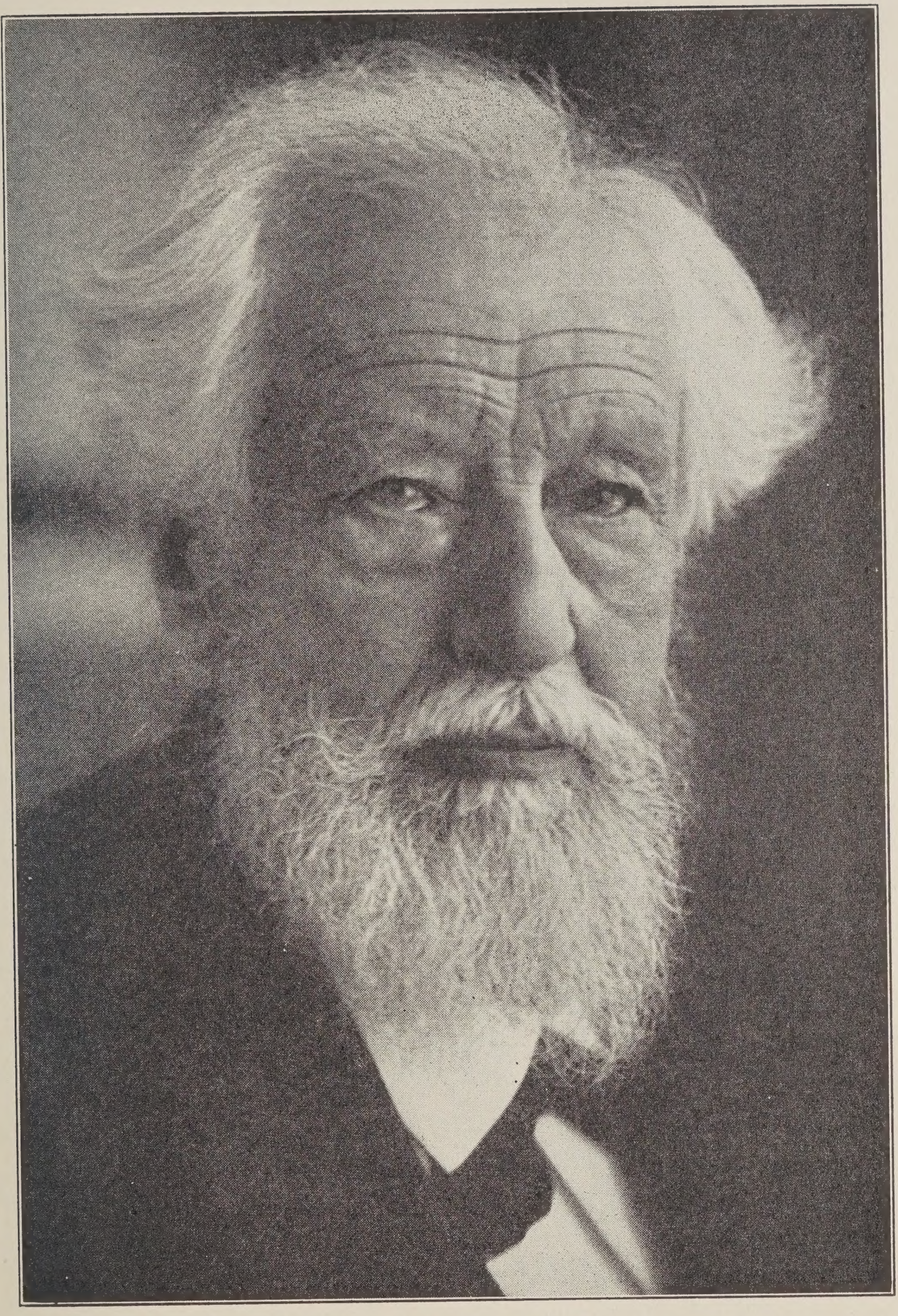

FIG. 27. Hugo de Vries, 1848-1935.

pheres of osmotic pressure when dissolved in 1 liter of water. So much for theory.

Pfeffer found that a 4 per cent solution of cane sugar at 15°C has an osmotic pressure of 208.2 cm of mercury. At 0°C this would be 197.4 cm of mercury. A 4 per cent solution of sugar is 100/855 of a molar solution; therefore, 100/855 of the theoretical osmotic pressure of a molar sugar solution (22.4 atmospheres or 1,702.4 cm of mercury) should equal Pfeffer's experimental value of 197.4 cm of mercury for a 4 per cent solution. The value thus calculated is 199.1 cm of mercury; a very close agreement to the experimental value of 197.4. Since Pfeffer's day, results in still better agreement have been obtained. The first law of gases, therefore, holds for solutions.

Van't Hoff found some discrepancies; indeed Pfeffer had noted them before him. If equimolecular solutions of salt and sugar are measured, it is found that, instead of having like osmotic pressures, as they should have for each has the same number of molecules, the salt solution has a higher pressure. Van't Hoff added a factor *i* to his equation to account for the proportion between the excess number of osmotically active particles and the total number of molecules known to be present in the solution. This factor took care of the divergence of electrolytic solutions from the perfect gas laws, but it told nothing about the cause of the divergence.

The Swedish chemist, Arrhenius, who later was a student of van't Hoff, explained the need of the constant *i* on the theory that salt molecules break down, or *dissociate* into their respective ions, when in solution. There thus arose the theory of the *dissociation of electrolytes*, one of the most important contributions to physical chemistry; indeed it may be said to have definitely established physical chemistry as a distinct science.

The plant physiologist Hugo de Vries (Fig. 27) applied the findings of Pfeffer and van't Hoff to living plant cells. By using the cell not as an osmometer, or instrument for measuring osmotic pressures directly, but as a "null" instrument, de Vries ascertained the osmotic pressure of a number of solutions, among which the following are examples:

	Osmotic Pressure in Atmospheres
1 per cent cane sugar	0.69
6 per cent cane sugar	4.05
1 per cent glucose	1.25
1 per cent potassium nitrate	3.50
1 per cent ammonium chloride	6.67

The agreement with theoretical values is very good; thus, the calculated osmotic pressure of 1 per cent cane sugar is 0.67 atmospheres. (Values beyond the first decimal place are affected by temperature.)

One of the neatest experiments in osmosis done by de Vries is the following:

Chemists were considering the probable formula of the sugar raffinose. There were three possibilities: $C_{12}H_{22}O_{11} \cdot 3H_2O$, $C_{18}H_{32}O_{16} \cdot 5H_2O$, and $C_{36}H_{64}O_{32}$, with the respective molecular weights of 396, 594, and 1188. By using the living plant cells of *Tradescantia discolor* as osmotic systems for determining molecular concentration, de Vries found those concentrations of sucrose and raffinose which were isosmotic with the plant cell. The method was that of plasmolysis. A sugar solution which will permit water neither to enter nor leave a cell is isosmotic with the solution in the cell; and if two sugar solutions are isosmotic with the sap of a plant cell they are isosmotic with each other and therefore equimolecular. De Vries found that on an average 6.22 per cent of cane sugar just barely plasmolyzes the *Tradescantia* cell and he found the same to be true of a 10.8 per cent solution of raffinose.

If the total weight of raffinose in a solution which is isosmotic with a sucrose solution is known, it is an easy matter to calculate the weight of one raffinose molecule from the following simple proportion:

$$\frac{\text{Total weight of raffinose}}{\text{Weight of one raffinose molecule}} = \frac{\text{total weight of sucrose}}{\text{weight of one sucrose molecule}}$$

On the basis of de Vries' findings, the following proportion should hold—converting per cent to grams per liter so as to be comparable to molarity:

$$\frac{0.108}{x} = \frac{0.062}{342}$$

then x, the molecular weight of raffinose, is 593.8.

Although the botanist would prefer using a plant cell as an "osmometer," the experiment is more simply, and slightly more accurately, done by finding that concentration of raffinose which is isosmotic with 3.42 per cent cane sugar, one solution being within and one without an osmotic sac. The value thus found for raffinose is 5.957 per cent. A 3.42 per cent solution of sugar simplifies the mathematics, for then:

$$\frac{5.96}{x} = \frac{3.42}{342}$$

and x, the molecular weight of raffinose, is 596.

Of the three possibilities of the chemical constitution of raffinose given by the chemists, one has a molecular weight of 594, viz., the second formula, $C_{18}H_{32}O_{16} \cdot 5H_2O$; the slight difference of 2 is due to unavoidable experimental error.

Osmosis involves the diffusion of water through a membrane; there are, therefore, as many kinds of osmosis as there are means of bringing about the movement of water through membranes or capillaries. The method so far considered rests on the difference in concentration of two solutions on opposite sides of a semi-permeable membrane.

The passing of water through a semi-permeable membrane can also be accomplished by establishing a difference in temperaure between the two sides. If a water-permeable membrane separates pure water from pure water, and if the temperature on one side of the membrane is raised, the molecules on that side of the membrane will become more active and will pass through from that side faster than they do from the cooler side. *Thermal osmosis* results.

If a membrane permeable to water separates pure water from pure water, and an electric potential difference is established between the two sides of the membrane, then the water moves from the one (electropositive) to the other (electronegative) side. This is *electroendosmosis.*

One obvious function of osmosis in the plant is the entrance of water and its passage from cell to cell. Whatever difficulty there may be in regard to an interpretation of the passage of water up stems (page 71) there is no question as to the entrance of water into living root hairs by osmosis. This fact alone places osmosis among the foremost of physiological processes. But there are other functions; among these is the maintenance of the turgidity of plant parts, notably leaves. Were leaves not turgid, they would collapse, as do wilted leaves. Woody stems possess a rigid framework of cellulose, but succulent stems, such as those of most cacti, maintain their form and rigidity because of turgor due to the osmotic pressure of the cell sap.

A highly specialized function is performed by the osmotic mechanisms of certain tissues which bring about the movements of leaves and other parts in response to darkness, shock, and other stimuli (pages 269, 270). The pulvinus (Fig. 80) of the sensitive plant probably functions by the exosmosis and endosmosis of water, exomosis bringing about collapse of the mechanical tissue and endosmosis reestablishing turgidity.

The mechanism of glandular activity has long baffled scientists, electroendosmosis having been resorted to as an explanation (see page 20). Glands secrete aqueous solutions of salts and other substances. Common examples of glands in the plant world are *hydathodes* (the analogue in the animal world is the sweat gland). The secretion of water by hydathodes is known as *guttation. Nectaries* are glands which secrete sweet solutions. Plant glands are osmotic machines in so far as the secretion of water is concerned, but the exit of materials is not osmotic.

Ionic concentrations produced against a gradient occur in plants and animals; for example, there is but 0.05 per cent of urea in the blood of man, yet the urine contains 2 per cent. Certain dyes when allowed to perfuse into the liver of a frog in very dilute concentration are given off 400 times more concentrated than the original solution. Plants also build up concentrations against a gradient. When slices of potato are placed in a bromide solution, the concentration of bromine in the living cells is increased 1000 times over the external solution. The marine alga, *Valonia,* stores potassium in its large central vacuole to the extent of 43 times the concentration of this element in sea water (page 129). It is questionable whether or not these examples of concentration of material in plants and animals are simple cases of osmosis. More than the diffusion of ions may be involved. Complex chemical reactions are possibly concerned.

IMBIBITION

The entrance of water into the plant is not due wholly to osmosis though this is the chief process involved. *Imbibition* also plays a part. Protoplasm takes in water by imbibition. In general, imbibition is the taking in of water by gels, and consequent swelling. Most organic gels, such as cellulose, agar, gelatine, and starch, swell when placed in water. Although attempts have been made to regard imbibition and osmosis as identical in so far as the forces involved are concerned, yet physiologists, concerned as they are with the swelling of gels on the one hand and the turgor of the cell vacuole on the other, regard the two phenomena as distinct because the respective systems in which they take place are mechanically, i.e., structurally, different.

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CHAPTER VII

THE ASCENT OF WATER

The mechanism by means of which water ascends a tree has long been one of the great problems in plant physiology. Although no final solution has yet been found, most of the hypotheses advanced have some convincing features.

Were it possible first to ascertain the path taken by the ascending water in a plant, the mechanisms concerned could be more satisfactorily considered. The great length of xylem tubes, formed by loss of the end walls between elongated cells, suggests that these function as miniature pipe lines for the conduction of water. A simple experiment performed two-hundred years ago proves that the principal upward movement of water is in the wood, i.e., inside the cambium ring. When a girdled twig is placed in water, the leaves remain fresh because they are receiving water which is coming up the wood. This must be true, for the bark, consisting of cork and phloem, has been removed. If the wood is removed and the bark left, the leaves soon wilt, showing that though phloem is available for the upward conduction of water, such conduction is inadequate if not totally lacking.

Having demonstrated that the ascent of water in plants takes place within the wood, the question arises as to whether the young and living cells or the dead xylem tubes are responsible. A definite answer cannot be given. In the past, it has been assumed that the dead xylem vessels conduct water. A route through dead tubes will involve an entirely different mechanism than one through living cells. The large size of xylem tubes formed in the spring compared with the small size of summer xylem may possibly have a bearing on the problem. The evidence, however, is by no means easily analyzed. The assumption has been that the distention of young wood is due to the upward rush of sap in the spring; but if the ascent of fluid took place in dead tubes it could not be effective in causing distention, for old cell walls are rigid. If the distention takes place in young cells, the responsible force must be osmotic pressure, for young cells are alive. Upward movement of water is greatest in summer when transpiration is at its height with full-grown leaves and well-developed stomata. In springtime, leaves are young or in the bud. Furthermore, increased transpiration will not involve excess pressure, for pressure is then replaced by tension owing to the rapid rate at which water is being removed, thus leaving none for the production of distention. Such a situation will mean that xylem cells formed at the time of maximum transpiration will be small, not large. *Rate* of movement is one thing, and *condition* of movement is another.

The misconceptions which have arisen in regard to springtime pressures and large spring vessels are probably due to the knowledge that sugar maples and some other trees "bleed" profusely when cut or tapped in early spring. This cannot be due to a rush of water and food from the roots because at sugaring time, in the north, the ground is frozen and no water can enter from the soil. Furthermore, the buds have not yet opened so there is no transpiration; and, finally, sugar is not stored only in the root of the maple tree. Pressure is there, but no pull due to a transpiration stream. The pressure is in living cells, primarily of the phloem where the sugar is stored, whether in stem or root. It manifests itself in various ways, by internal "bleeding," by a general movement of stored sugar, by bursting the bark and producing "frost-cracks," and by external "bleeding" when the tree is tapped. The nature of this pressure is discussed at the close of this chapter.

The distention of young xylem vessels is best interpreted on the following basis. In spring there is little or no inward pressure exerted by the bark; the newly formed xylem cells can, therefore, readily expand osmotically. The summer addition of wood stretches the bark considerably and thus exerts great pressure. Consequently, young xylem cells formed in late summer cannot expand and so remain small. If a tight band is placed around a tree, small cells like those in summerwood are formed in the spring; if the bark is slit in summer, springwood is formed. Fruit growers, indeed, often slit the bark of trees to relieve pressure. Distended spring xylem is, therefore, no proof that water ascends a tree by way of the dead xylem tubes.

The condition of the xylem in some trees, such as the locust, *Robinia,* is such as to indicate that liquid rises in living cells. The older wood vessels are full of tyloses or proliferated tissues, which block the passage of water; the tubes are, therefore, incapable of conduction. The sap, as a consequence, must ascend by cells less than a year old, that is, by living cells. These are important considerations but still not conclusive proof that water in all cases rises only in living cells.

As the precise path taken by upward moving water in the plant is not known, the problem of the responsible mechanism will have to be considered from every possible point of view.

The mechanism may involve forces operative in dead capillary systems, or forces operative in living systems. The former include static forces, which merely hold water in position, and mechanical and electrical forces which move water under pressure or under tension. Forces operative in living tissue include slow movements of water under pressure.

The various types of mechanisms and the forces they involve may now be considered.

Capillarity as a force involved in the ascent of water is readily disposed of by comparing the heights to which water will rise in tubes of diameters equivalent to those of xylem vessels with the required height to which water must rise in trees. Water rises only a few inches or at most one or two feet in capillaries of diameters comparable to those of average xylem vessels. Were xylem tubes of the order of 10 mμ (0.00001 mm) in diameter, capillarity would carry water to the tops of the tallest trees. But xylem tubes average 150 μ (0.05 mm) with maximum sizes equalling 0.4 mm in tropical lianes. The height water will reach in tubes of such diameters is insignificant compared with the height of a tall tree.

There is, however, a more serious criticism of capillarity as a force operative in the ascent of sap. Xylem ducts are full of water, and were full of water at the time they were formed. The problem, therefore, is not how to fill an empty tube. Furthermore, xylem tubes are closed at the bottom and top. No capillary will function under these conditions. Capillarity operating in xylem ducts thus apparently cannot be regarded seriously as an explanation of the ascent of water in plants. There is, however, another seat of capillary forces, namely, the cellulose walls of wood, which imbibe water.

Imbibition is a force certain to be operative to some degree in conducting water up a stem, for wood soaks up water with avidity. Sachs was the first to interpret the ascent of sap on the basis of capillarity in cell walls. The only severe objection to the imbibing qualities of cellulose as an important factor in the ascent of sap is rate of movement. Absorption in wood is too slow to account for the comparatively rapid ascent of water.

Static forces such as capillarity and imbibition are, then, important in maintaining the position of water in the plant, but cannot cause movement of fluids in sufficient quantity and at a sufficient rate to account for the ascent of sap in trees.

Atmospheric pressure as a force conceivably contributing to the ascent of sap may be immediately discarded because, although it will raise water 34 feet under ideal conditions, it would exert almost as much pressure at the upper or leaf end of a xylem tube as at the lower root end, were it actually operative at either end. It is not effective because both ends of xylem ducts are closed; in any case, it cannot exceed 34 feet.

Electroendosmosis (page 20) is a well-established property of certain types of capillary systems and, if applicable to one type, it may be to another. Its two prerequisites, capillaries and an electromotive force, may both be present in plants, but there is indubitable proof of only one, the capillaries. The electroendosmotic flow of water through wood can be demonstrated experimentally. If there is an electromotive force present in plants or their environment, of sufficient strength to bring about an osmotic flow of water through wood capillaries, then there is no reason why this form of energy should not be responsible for the ascent of sap.

It must first be ascertained if electrical forces are present in plants. That they are indeed present appears likely from the work of Lund on potentials in plants; but the differences in levels of electrical energy are probably insufficient for electroendosmotic flow—a difference in potential of 400 millivolts between the stem and root of *Fuchsia* has been measured. However, the electromotive force need not be within the plant. If the air is charged in relation to the earth, as are clouds, there will exist a field of static electricity, and this may exercise an influence.

Transpiration, or the escape of water from leaves through stomata, is a force contributing to the rise of water in plants; this is suggested in the expression *transpiration stream.* Evaporation lowers the water content of leaf tissue near the surface; this exercises a "pull" on the water supply beneath the surface which in turn draws from the capillary water columns in the xylem tubes. This is the basis of the *cohesion theory* advanced by Dixon and Joly. The water in the conducting tubes of xylem is assumed to exist in unbroken columns. Transpiration results in a deficiency in water in the cells of the leaf which is compensated for by the osmosis of water from adjoining cells, and these, in turn, draw from the xylem elements of the leaf. One important condition of the theory is that the water column must remain unbroken; this is thought to be possible because of the capacity of water to resist tension, i.e., a column of water possesses tensile strength.

The assumption on which the foregoing hypothesis rests, namely, that the column of water shall not be broken, must be considered in the light of an experiment by Strasburger. He cut off a tree from its roots and swung it over into a tub of dye. On making sections of the upper stems some hours later, he found dye in the tissues. An upward flow of solution had, therefore, taken place even though the columns of water in the xylem tubes were apparently broken. Possibly the cut ends of the tubes were merely shortened and the water column not destroyed. There is another possibility; the fine water columns in the pores of the cellulose walls may have remained intact. If the water of imbibition in the wood remains continuous, and as the distance the water would have to travel around the larger broken columns in the xylem tubes to the intact water columns is not great, then the cohesion hypothesis would not be severely weakened by Strasburger's experiment.

The cohesion hypothesis is given support by the simple experiment illustrated in Fig. 28A and duplicated with an artificial apparatus in Fig. 28B. The rise of the mercury demonstrates that evaporation, whether taking place from leaves, animal membranes, or artificial porous material, will raise a water column. The magnitude of this force is great and must be met by an equal one represented by the cohesion of the molecules of the water in the unbroken columns.

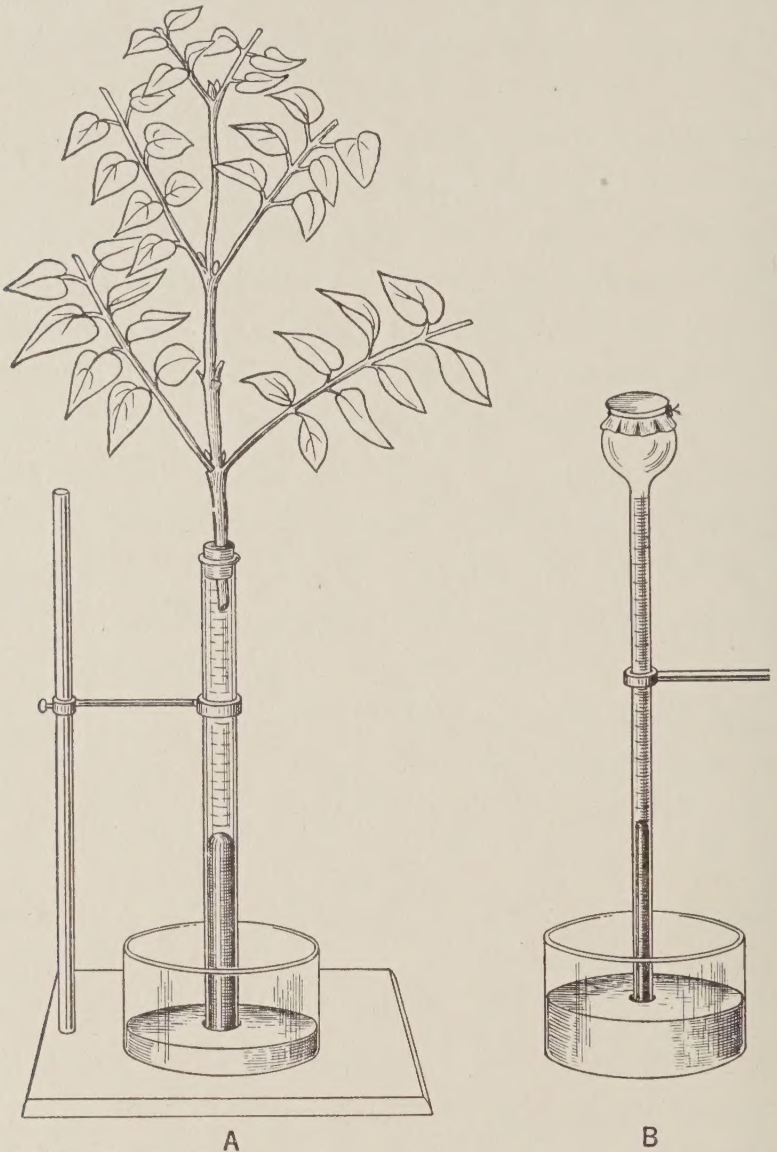

FIG. 28. A. Rise of a mercury column due to transpiration from leaf surfaces. B. Rise of a mercury column due to transpiration from an artificial membrane. (After Palladin.)

That transpiration exerts an actual pull and does not merely displace water is shown by experiment, for it is possible to raise a mercury column to 90 cm. This is considerably above that which atmospheric pressure can accomplish alone. An atmometer (Fig. 31) or a plaster-of-Paris surface will accomplish this; even an animal membrane will raise a mercury column to a height above 76 cm. Such experiments demonstrate the reality of the lifting power of transpiration.

If, now, there is any other pressure at work, such as root pressure (see page 79), it will contribute to, and may become the primary force responsible for the ascent of water in plants when transpiration is at a minimum, as on humid days, especially in tropical rainforests where transpiration is low.

After being in favor for a number of years, the cohesion theory of the ascent of water has met with severe opposition. Priestley expresses his objection to the theory because of its failure to give evidence of a pressure in water-conducting tissue. A water column under tension, hanging, as it were, from the highest trachea in a hundred-foot tree, can obviously not exert lateral pressure; and pressure is needed to account for a variety of phenomena such as bleeding. This criticism is met, however, by James and Baker, who point out that there are two separate systems in the plant, the transpiration stream and the slow movement of sap due to pressure which is responsible for such phenomena as bleeding.

All mechanisms so far considered which might be involved in the ascent of water in plants have to do with forces operative in dead systems. The slow movement of water under pressure in the plant takes place in living cells. That this is true is shown by the fact that such movement is stopped by anesthesia.

In general, all forms of slow movement of fluids in the plant are known as *sap pressure*, but there is much confusion between the various types of sap pressures and between slow movement in living cells and the rapid movement of the transpiration stream in dead tissue, if that is its route. The issue is further confused by the fact that sap pressures may contribute to the forces involved in the transpiration stream.

The forces responsible for the slow movement of sap in living tissues are the following. *Osmotic gradients* will account for the movement of water from one cell to another if the gradient is maintained. If it is to account for the transpiration stream, the gradient must also be in the right direction. For osmotic movement to occur along a chain of cells there must be entrance of water by endosmosis at one end and exosmosis at the other end

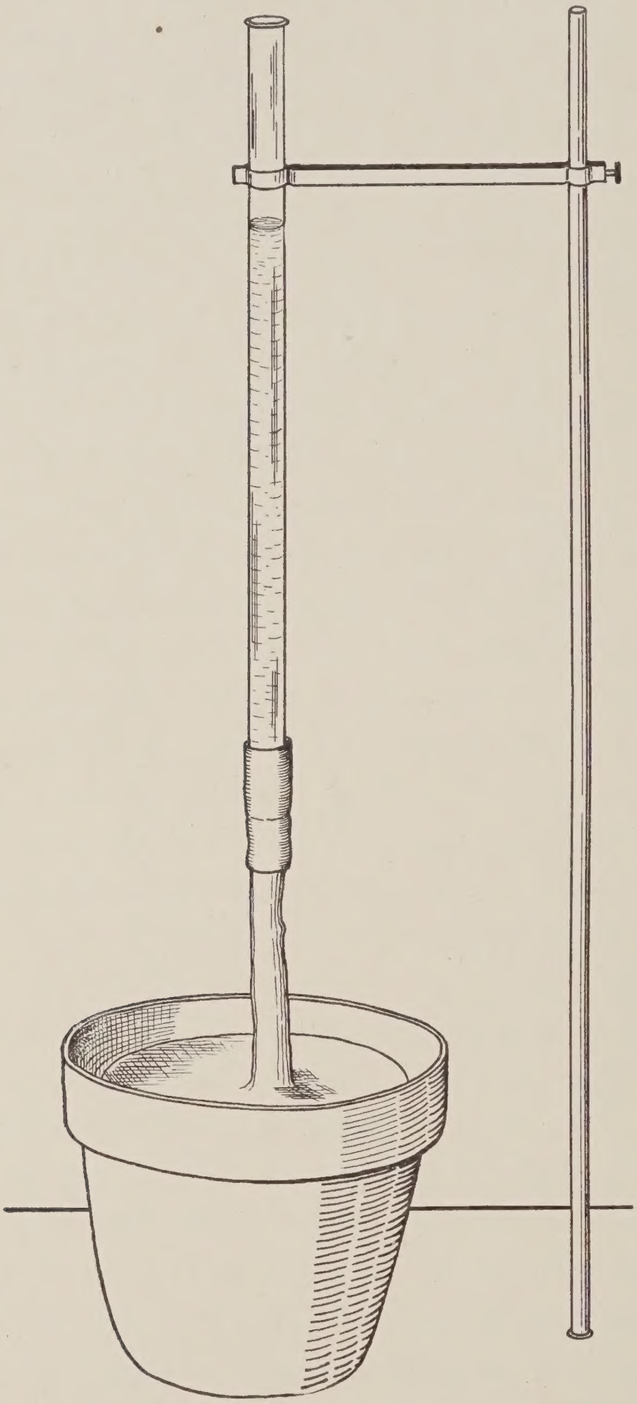

FIG. 29. A simple manometer for demonstrating root pressure.

of every cell in the chain. For these to occur simultaneously there must exist a difference in concentration of fluids at the two ends of the cell, either of the sap within the cell or of the sap in the two adjoining cells. The former situation is unlikely, especially where protoplasm is actively streaming, for it could not be maintained. Differences in the concentration of sap in adjoining cells very probably exist. Throughout the plant and animal kingdoms there are *gradients*, metabolic gradients, acidity gradients, concentration gradients, and others. Of three adjoining cells, the lowest one, nearest the root, is likely to be the least concentrated in salts, the center one more so, and the upper one most. Such a system will permit the upward movement of water osmotically through living cells.

That there actually is a salt concentration gradient, and in the direction as just postulated, may be deduced from the fact that salts are continually carried to leaves where they cannot go farther. They thus pile up there, and, as they are at the same time constantly being taken from the root, they are least concentrated in the latter tissues. A salt concentration gradient is an osmotic gradient, which must inevitably mean a movement of water from the least to the most concentrated cells, and this is from root to leaves.

Root pressure (Figs. 29, 30) has been the subject of lively discussions in botanical science. Additional proof of its existence, if it is needed, is to be had in recent work by Philip R. White. Former doubt cast upon the reality of root pressure may have been due to failure to recognize the essential unity of several types of pressure and forms of tissue turgor which have been variously named. It is not certain that all forms of sap pressure are identical; indeed, the indications are to the contrary. Thus, bleeding may be momentary or continuous and is not polarized, i.e., not unidirectional, whereas root pressure, exudation pressure, and guttation (page 89) are polarized, i.e., always in one direction. For example, if a stem is cut free from the plant, then exudation through living cells occurs only at the morphologically upper end, although the ducts are open at both ends. Several other pressures, supposedly different and variously named because they were observed in connection with different manifestations, may be identical in origin.

"Bleeding" often results when a plant is cut. It may be momentary, in which case it is probably due to tissue turgor, or it may be continuous. If it is continuous, the amount of exuded matter may be so great as to exceed the volume of the tissue which has supplied it, thus showing that the pressure responsible has not immediately expended itself but is maintained for a time. This suggests that it is not a water sac under pressure which has been punctured, but rather a supply maintained under pressure. The nature of this pressure has been the subject of investigation ever since it was discovered by Stephen Hales in 1727 (Fig. 1).

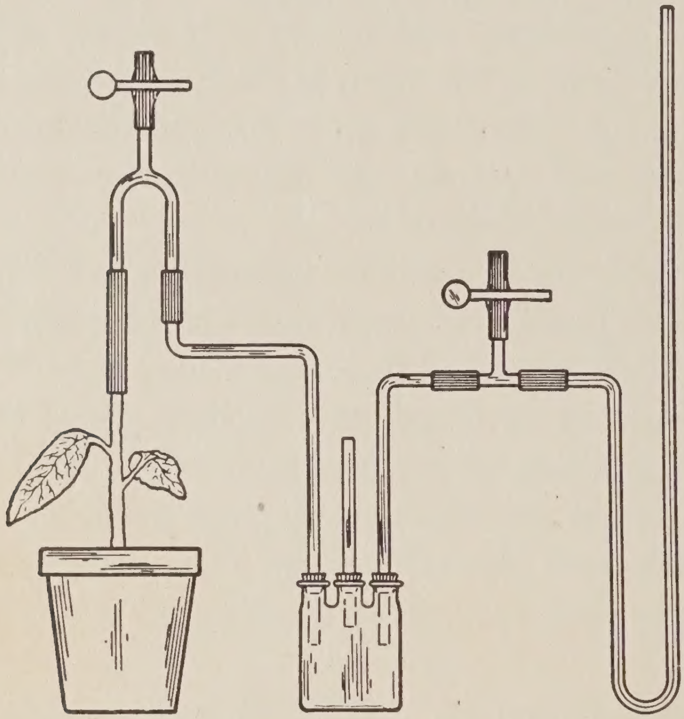

FIG. 30. Sap pressure gauge, designed to measure root pressure, or the suction set up by transpiration from a branch, as in Fig. 28 A. Woulff bottle and vents facilitate adjustment of mercury level and release of entrapped air bubbles.

Bleeding is sometimes of considerable economic importance. The tapping of the sugar maple in spring; the daily cutting of rubber trees (*Hevea* and *Ficus*) in the tropics; the bleeding of palms (*Areca* and *Caryota*) which yield sap used by the natives to make "toddy" or palm "wines"; the tapping of the southern longleaf pine (*Pinus palustris*) for turpentine; and the cutting of the century plant (*Agave mexicana*) for mescal and pulque (the fermented juice) : these are all examples of plant bleeding of economic value. Other familiar instances are the bleeding of "milk" weed and of "blood" root. Still another case, which however, may not be bleeding as here interpreted, are the "water vines" of the tropics, species of the genus *Vitis*, which leak profusely when cut.

Bleeding is a manifestation of pressure, and has been ascribed to *root pressure* or *exudation pressure*. As these have to do with *sap pressure*, this, too, is an applicable term. If these pressures, which may be one and the same, are responsible for the movement or translocation of food (page 176), they may then be called *translocation pressure*. Finally, there is so-called *suction pressure*, not a fortunate expression as suction implies the absence of pressure; nor is it quite clear what pressures or tensions are to be included. If the term is at all applicable, it must relate to the transpiration stream rather than to exudation.

The situation may be simplified by recognizing two types of forces responsible for all movements of materials in plants. The one type is responsible for those various bleeding and exudation phenomena just enumerated, and may, when necessary, contribute to the ascent of water; the other type is primarily responsible for the transpiration stream.

In summarizing their research on sap pressure and sap movements, W. O. James and H. Baker conclude that, as the sap exuded in bleeding does not come out of xylem vessels but out of living cells, then it is sap pressure or hydrostatic pressure which is responsible for all sap movements, except the transpiration stream.

That all sap movements, the transpiration stream excepted, are due wholly to one and the same force was not the opinion of Molisch, nor is it accepted by some modern workers. Stephen Hales defined root pressure as the force by which the roots drive sap upward in a continuous stream in the absence of a transpiration stream. How that force is developed he did not know. Some present-day workers regard non-polarized momentary bleeding as due to cell turgor, and polarized exudations as due to pressures in which turgor plays a necessary correlative role but not a causal one. Sap movements within the plant belong to the latter category. Osmotic phenomena, electroendosmosis, or energy derived from respiration may be responsible. The last suggestion was originally made by Pfeffer and elaborated by Blackman.

Philip R. White has been able to measure directly the pressures developed in roots grown in culture (page 240). By attaching a manometer, or gauge, to a single root growing in nutrient solution he has measured secretion pressures of more than 6 atmospheres. Flow continues as long as the root can be kept in an aseptic condition, two weeks being the longest time so far achieved. The flow is not uniform, having a peculiar day-night rhythm, which is at present not understood. Mercury and water manometers, and an opposing air pressure were used; with mercury columns about 2000 mm. were obtained.

By opposing the root pressures with compressed air it was possible to estimate the pressure required to stop exudation of sap from the root. A compressed-air pressure of 90 pounds, or 6 atmospheres, did not appreciably alter the regularity of the flow. Such pressures appear, therefore, to be significant in comparison with the pressures actually developed by the roots. When it is realized that 6 atmospheres will raise water nearly 200 feet and that such pressures are developed in single roots of a tomato plant which never reaches a tenth of that height, the importance of this force in the movement of sap can be appreciated.

White points out that turgor cannot be wholly responsible because it will not maintain a continuous flow. As already mentioned, when a plant is punctured, it is not a sac under pressure but a more permanent source of pressure which is tapped. Turgor can only produce exudation momentarily. The magnitude of the force developed and its potential importance in the rise of sap were probably missed by earlier workers, because the rate of flow is so much slower than that of the transpiration stream as to be masked by the latter.

The force responsible for root or exudation pressure remains unknown, but there is general agreement on its magnitude and significance. It is not wholly responsible, however, for the transpiration stream nor the mass upward flow of soil water.

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CHAPTER VIII

THE USE, LOSS, AND STORAGE OF WATER

USE

As a Solvent. Active plants and animals are about three-fourths water. Water serves numerous functions in the plant, its chief use being as solvent of salts, sugars, and other materials. This complex solution is the dispersion medium of protoplasm. It is the substratum in which cellular reactions take place.

Whereas the proportion of water in plants is about three-fourths of their total weight, it varies widely from a minimum of 10 to 15 per cent in seeds to 96 per cent in some succulent tissues. There are records of even lesser and greater amounts, though they are rare. Some values in percentages are: cucumber fruit 96, carrot root 90, apples 83, wheat grain 14, and oat grain 11.

Photosynthesis. A second important role of water in the plant is its use in photosynthesis (page 133). The first step in the formation of organic matter from inorganic material is the union of water and carbon dioxide. The amount of water thus consumed is considerable, though to be sure not nearly so great as the amount taken in by roots or that lost by transpiration.

The use of water as a solvent and its use as an ingredient in the synthesis of sugar represent its chief roles in the plant, but there are several other minor ones.

Transpiration. The amount of water required by a plant may be defined as "the ratio of the units absorbed to the units of dry matter produced" in a growing season; though such a definition loses sight of the water needed when no dry matter is being added. The units absorbed far exceed those incorporated in the plant. The rest is lost through transpiration, mostly through the leaf pores known as *stomata.* A large oak may give off as much as 150 gallons of water a day, and a single corn plant 54 gallons in a growing season. It has been calculated that each pound of vegetable matter produced by a plant means a loss of 40 gallons of water.

Transpiration is less at night and less on a cloudy day than in sunshine. Low humidity, intense light, high temperature, wind, and rarefaction of the air favor transpiration. High humidity reduces transpiration.

In order to have a standard of comparison for water lost by transpiration, Livingston developed the *atmometer* which is a porous thimble made of kaolin (Fig. 31). By means of it one can obtain an accurate measurement of the evaporating power of the air. The atmometer replaces the old and more cumbersome method of evaporation from a free water surface in a tank.

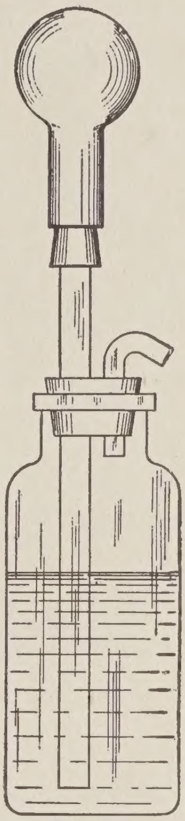

Fig. 31. A porous clay atmometer, Livingston model.

Excessive transpiration causes wilting if the water lost exceeds that taken in by the roots. A humid environment obviates the need of protection against excessive transpiration; consequently, tropical plants which lack protection show signs of incipient wilting on sunny days. Many tropical herbaceous plants, which normally grow in moist situations, will die if left unwatered in the bright sun for a single day.

That water is given off by a plant may be demonstrated by placing a potted plant under a bell jar; in time, water is deposited on the inner surface of the jar (Fig. 32 A, B). The effect of light on transpiration may be demonstrated by growing corn seedlings, some in darkness, some in diffuse light, and some in bright light, and measuring the water loss. They lose water in the ratio 1 : 3 : 8 (other conditions being constant). Plants contain abundant water at night, but may show a water deficit in the day. Control of transpiration does not depend solely upon a plant's surroundings. Conditions existing within the plant also play their part, but of them little is known, and they change with the age of the plant.

The startling conclusion from the above facts is that a plant does not make direct use of all the water absorbed, but loses the bulk of it; this appears to be a needless waste. Perhaps the plant is not a very efficient machine. Before drawing this conclusion, it will be well to seek a possible benefit such as the intake and ascent of salts.

Early statements on the relationship between transpiration and the intake of salts were based on what seemed to be sound reasoning but later proved to be erroneous when experimentally tested, at least so it now appears. Pfeffer and older physiologists thought that the amount of salts taken in by the plant from the soil was proportional to the amount of water transpired; and this would seem to be true. Haberlandt, however, objected on theoretical grounds. He reasoned that as plants in the tropics,

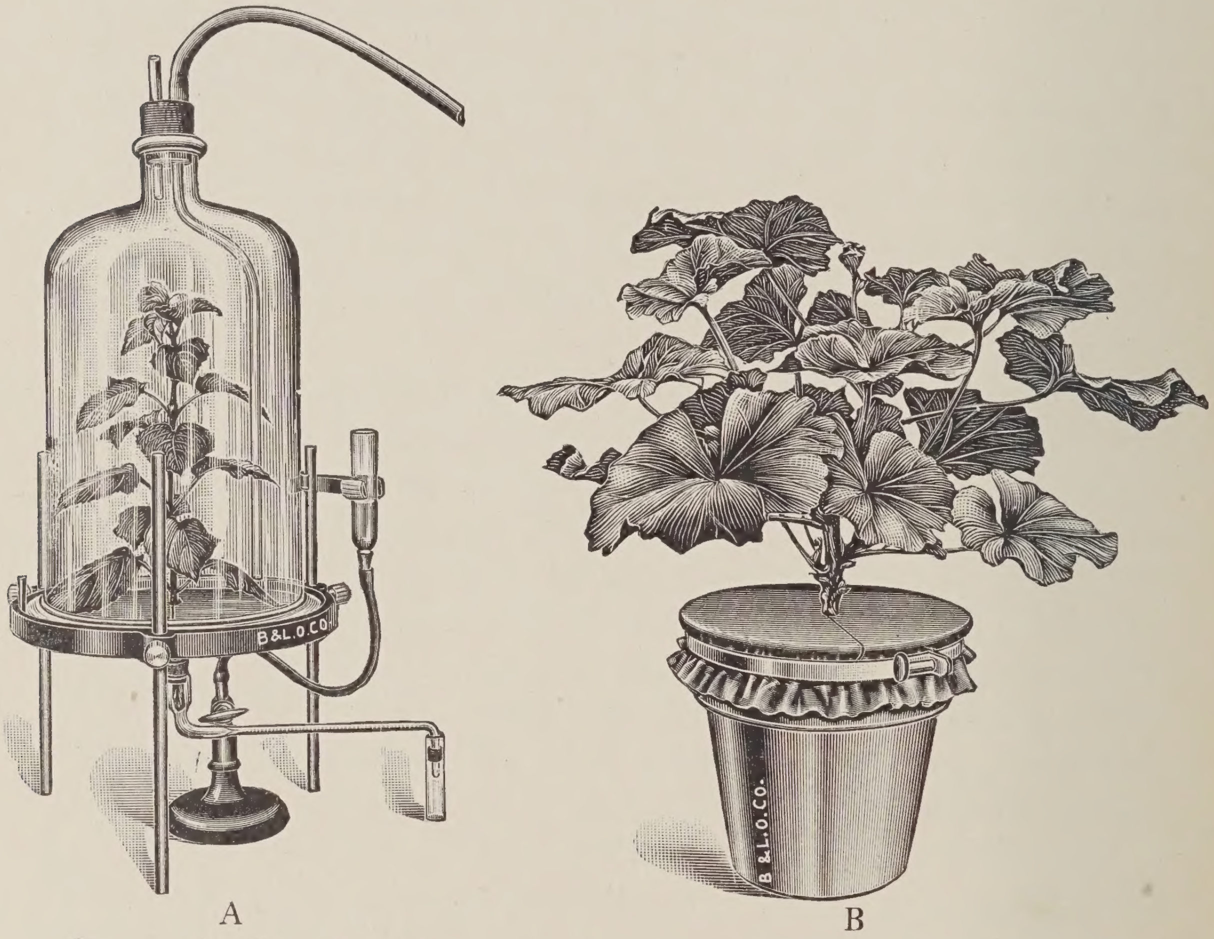

FIG. 32. A. Glass chamber for studying effects of gases on plants, for measuring gases taken in or given off, and for demonstrating and measuring transpiration. B. Flower pot in an aluminum shell with a rubber covering to prevent loss of water from the pot and from the surface of the soil, for determining transpiration when plant is enclosed in a gas chamber as in Fig. 32 A. (Courtesy of the Chicago Apparatus Co.)

where the humidity is very high, transpire but little compared with plants in temperate regions, the intake of salts should differ greatly, whereas this is not true. Later experimental evidence proved his reasoning to be correct. Plants grown in the sun may transpire a third more than those in the shade, yet the amount of ash may actually be less in the sun-grown plants. Solutes, therefore, apparently enter a plant independent of the entrance of water.

The foregoing conclusion does not mean that once within the plant, salts may not be more rapidly translocated by a quickly moving transpiration stream. That they are, is a conclusion difficult to escape, but they cannot be moved more rapidly than they are supplied to the stream and the rate of supply, i.e., the rate of entrance from soil into root, is determined by diffusion rates which are slow.

Regulation of temperature is still another suggested function of the transpiration stream and the excessive loss of water. Evaporation from a surface reduces temperature, and as plant temperatures usually follow the temperature of the air fairly closely (except at extremes) transpiration might help cool plants when the temperature of the air rises to harmful heights.

Curtis expresses the opposite point of view when he states that the transpiration stream is not only useless but harmful. The presence of waxy coverings and sunken stomata as protection against excessive transpiration suggests that the loss of water is a useless waste; but one wonders why the plant has developed ways of protecting itself from a harmful process rather than eliminating the process. There are but two possible deductions: either the true function of excessive transpiration has not yet been learned or it is an example of the inefficiency of nature. The second answer is by no means the more likely one, for transpiration may be highly significant in some way; it surely serves in aiding the absorption of carbon dioxide and the elimination of oxygen, both of which require that tissue surfaces be kept moist. A moist surface means evaporation, and therefore transpiration. Thus, in order to maintain one necessary condition, the plant is forced to be wasteful in another respect. No mechanism nor process in nature is perfect. All life is a compromise.

LOSS

Stomata. The water lost by leaves passes out by way of leaf-pores or *stomata.* Practically none can be lost by direct evaporation from the ordinary leaf surface because of a waxy coating or *cuticle.* Where a cuticle is lacking, as it is on some exceptional kinds of leaves, surface evaporation may be relatively high. The size of stomatal openings is determined by the two *guard cells,* which function osmotically. The exact time and cause of the closing and opening of stomata have long remained in doubt in spite of many careful studies, and it is still a question whether or not the problem has been wholly solved.

Although stomata are discussed here in connection with water loss, they possess another important function, namely, the control of gaseous exchange, in particular, the intake of carbon dioxide for use in photosynthesis (page 144). If stomata control transpiration, then one would expect them to close when the excess water lost is too great and therefore damaging. In certain instances this is true; thus, many xerophytes, or plants living in dry localities, close their stomata when they begin to wilt.

That stomata are responsible for water loss may be demonstrated by placing bits of hygrometric or cobalt-chloride paper on the upper and lower surfaces of a leaf. The paper against the lower surface with stomata turns pink in a few seconds, showing the presence of water, while the paper on the upper surface, which lacks stomata, remains blue.

Though stomata exercise some control over transpiration, they are influenced by light more than by moisture. Stomata are commonly open in daylight and closed at night, certain kinds reopening in bright moonlight.

Some workers have attributed stomatal movement to changes in permeability; thus, Maximov holds that increased permeability diminishes turgor, causing guard cells to approach each other and the stomatal aperture to close.

The hypothesis of stomatal movement now most widely accepted is Scarth's. He holds that hydrogen-ion concentration is the controlling factor, and this is altered by both photosynthesis and respiration. Through a reversible transformation of sugars, starches, and intermediate carbohydrates pronounced changes in osmotic pressure are produced. The effect of light is indirect; stomata open in the light because of an increase in alkalinity of the sap of the guard cells. Light intensity is thus a factor in stomatal movement only in so far as it affects photosynthesis. When light intensity is sufficient to overbalance, through photosynthetic activity, the production of carbon dioxide by respiration, then the stomata open. When, in darkness, respiration is more active than photosynthesis carbon dioxide accumulates, acidity rises, and the stomata close. Closure is due to decrease in osmotic pressure of the sap of the guard cells because of the formation of starch from soluble sugars which are produced by increased acidity.

If the foregoing chain of events is restated, but in the reverse direction, it becomes: bright light augments photosynthesis bringing about the use of and, therefore, a reduction in the respiratory carbon dioxide; the *p*H of the guard cells consequently rises to 7 or above; the alkaline sap accelerates the hydrolysis of starch; the sugars thus formed raise osmotic pressure; water enters, increasing turgor and cell volume; the guard cells bulge and the stomatal apertures enlarge.

Guttation. The secretion of water by plant glands known as *hydathodes* is termed *guttation.* The water appears at the surface in the form of large drops which when numerous give to the plant a striking appearance. When guttated water is given off in great quantity from large plants as in the tropics, the falling droplets present the appearance of a fine rain. Hydathodes, which are responsible for guttated water, are epidermal structures; they may be single cells, or mere openings between cells, or quite complex and highly specialized groups of cells. They do not exude pure water; salts and other materials are dissolved in the aqueous secretion.

Guttation is greatest when the air is moist; hydathodes apparently thus take care of excess water which cannot leave by transpiration, and so possibly assist in maintaining the rate of upward movement of water when transpiration is not sufficiently effective.

Protective Structures. In addition to stomata and hydathodes, both of which augment water loss, plants possess structures for conserving water. Though the great loss of water through stomata due to transpiration would seem to oppose the fact, yet plants are usually concerned in preserving their water supply, and develop special devices for doing so. Chief among these is the waxy cuticle which coats most leaves. The sticky covering on many bud scales is another type of protective layer preventing drying out. Scales may be waxy, resinous, or hairy; all these sorts of coatings protect against desiccation. Plants living in dry regions or in rarefied air, as in deserts and on alpine summits, have the characteristics of xerophytes, with structures preventing excessive transpiration. Structural features which protect against excessive water loss may be highly specialized; such are sunken stomata, mucilaginous cells, and reduced leaf surface. In a number of plants, e.g., the bluegrass, *Poa pratensis,* the leaf surface exposed is reduced by the rolling of the edges inward, or by folding of the leaf as a whole, thus greatly reducing water loss through transpiration.

STORAGE

Plants have numerous ways of collecting and storing water. The simplest is a pocket formed by leaves. This method is illustrated in *tank epiphytes*, which are bromeliads including the genera *Tillandsia*, *Nidularium,* and *Vriesia.* They are a conspicuous feature of the American tropics. Being high off the ground they have need of a reservoir to hold water. Tank epiphytes collect rain water, often as much as a liter to a plant.

In the climbing plant, *Dischidia rafflesiana,* certain of the leaves are formed into sacs or pouches. They collect water which is absorbed by aerial roots that grow into the leaf-like bag from the stem. Pitcher plants also collect water in leaf-like pockets (Fig. 83).

Succulents have specially developed tissues for the storage of large quantities of water. The fleshy leaves of *Sedum*, *Crassula,* and *Aloe*, and the stems of cacti are excellent water-storage systems. The spongy-leaved crassulas hold water with such remarkable tenacity that they retain their vitality and may sprout months after being collected and put away as herbarium specimens. The barrel cactus of Mexico and Arizona yields a plentiful supply of water if cut open and the tissue reduced to pulp.

Water reservoirs may be of living or dead cells or formed from intercellular spaces. When single cells serve as reservoirs, as in *Mesembryanthemum crystallinum*, a solitary epidermal cell enlarges into a large water bladder. Ordinarily, water-storage tissues are living; consequently, the water becomes part of the protoplasm, or at least of the sap, or the mucilage of the cells, as in cacti and the spurges. Sometimes water-storage tissues develop into conspicuous structures such as the pseudobulbs of epiphytic orchids.

From the above list of plants possessing water-storage tissues, it is evident that these specialized structures are developed primarily in plants that have need of them; this is notably the case in epiphytes and inhabitants of deserts.

The advantages of water storage are great where plants live in dry localities, or where rain falls but a few months each year. Plants accustomed to regular precipitation become so dependent upon it that even a slight deviation from the normal may be disastrous. The yield of vine crops is to a great extent determined by the distribution of rainfall. A variation of two inches in July will cause a variation of $13,000,000 in the value of the corn crop in Ohio.

Kinds of Water. Water as a solvent is "free water." Free water freezes. Water adsorbed or otherwise held to the surface of a hydrated particle is "bound" water. Bound water does not freeze. Organisms contain both free and bound water. Free water is readily given off, e.g., by transpiration. Water colloidally bound to proteins is not readily given off. Gortner and Newton found a correlation between winter hardiness (resistance to freezing) in wheat and the percentage of bound water. The winter hardiness of certain species may, therefore, be due in part to a greater quantity of bound water.

The discovery of heavy hydrogen brought to light at least two new forms of water; these are: H^2H^1O, and H^2H^2O (H^2_2O).

The symbol now generally used for heavy hydrogen is D, standing for *deuterium*; the original symbol, H^2, has some advantages, particularly in writing the formula for heavy water. The freezing point of H^2H^2O, or D_2O, which is *heavy water*, is 3.8° higher than that of ordinary water, and the boiling point 1.42° higher. It was first thought that heavy water would not support life, because tobacco seeds apparently would not sprout in it. However, other forms of plant life are variously affected by it. Heavy water retards the growth of bacteria, and accelerates spore production; but it seems to affect most higher plants little if at all.

The form in which water occurs in the plant must determine in part its role in the metabolism of the plant, but this phase of the general subject of the physiology of water is as yet little understood.

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CHAPTER IX

ACIDITY

It is only in recent years that the importance of acidity in the physiology of the plant has become evident. A treatise in plant physiology written before 1900 would have contained little or no discussion of this subject. At that time acidity was thought of in terms of *replaceable hydrogen* or *normality*. Not until the theory of molecular dissociation advanced by Arrhenius in 1883 had become well known was the importance of acidity as a physiological factor fully appreciated.

Arrhenius stated that strong electrolytes in solution dissociate into their component parts or *ions*. This explains why a normal solution of hydrochloric acid is a poison whereas a corresponding solution of acetic acid is a weak vinegar, suitable for use as a food. Both are of equal value in terms of normality or replaceable hydrogen, but not from the point of view of *free hydrogen ions*. Physiological acidity, or that acidity which determines whether or not an acid tastes sour, is largely a question of hydrogen ions and not of replaceable hydrogen. Hydrochloric acid dissociates almost wholly into H^+ and Cl^- ions when in solution. That it is fully dissociated when of moderate concentration is the newer point of view. Acetic acid, on the other hand, is but slightly dissociated, so that the number of free H^+ ions is small in proportion to the number of undissociated molecules, from which the so-called *dissociation constant* is obtained. If the dissociation constant is designated by K, then

$$K = \frac{[\text{anions}] \times [\text{cations}]}{[\text{molecules}]}$$

For acetic acid, this becomes,

$$K = \frac{[CH_3COO^-] \times [H^+]}{[CH_3COOH]}$$

the numerical value of which is 1.86×10^{-5}; this indicates that acetic acid is 0.4 per cent dissociated.

Although it is now recognized that strong electrolytes are fully dissociated even up to normal concentration, yet, reasoning for the moment along the lines of former hypotheses, a molar solution of nitric acid is 82 per cent dissociated, whereas acetic acid is but 0.4 per cent dissociated, which means that only 43 molecules dissociated out of every 10,000. The salt, potassium chloride, is 86 per cent dissociated at a concentration of 0.1 M; this means that in a 0.1 M solution (about 0.7 per cent) 86 out of every 100 molecules of potassium chloride are dissociated into their two ions, K^+ and Cl^-, whereas 14 molecules remain intact.

The hydrogen molecule consists of but one electron and its nucleus; it is, therefore, on ionization reduced to the unique condition of a nucleus devoid of electrons, and as such becomes the smallest and fastest-moving of ions.

Hydrogen ions are responsible for acidity; therefore, their concentration and activity are a measure of acidity. The hydroxyl ion, OH^-, is responsible for alkalinity. When the concentrations of H^+ and OH^- ions are equal, the solution is neutral. This is the situation in pure water. As water is a weak electrolyte and dissociates but slightly, the concentration of H^+ and OH^- ions in it is very small. The amount of the H^+ ions in neutral water is 0.0000001 grams per liter. The amount of the replaceable hydrogen in a normal solution of any acid is 1 gram per liter. If the acid is a monovalent one such as HCl, and fully dissociates in solution, then 1 gram is the weight of the free hydrogen ions in a normal solution. The amount of the hydrogen ions in a normal solution of any fully dissociated base, such as sodium hydroxide, is but 0.00000000000001 (10^{-14}) grams. To handle such figures would be very cumbersome; logarithms, therefore, are used. Acidity is expressed in terms of the logarithm of the hydrogen-ion concentration, and, as this concentration is a decimal, the logarithm is negative. The symbol used for this negative logarithm is *p*H. H is the hydrogen and *p* is the initial letter of the German word "Potenz," which means potential in the sense of logarithm or exponent. The *p*H of a solution is then the negative logarithm of the hydrogen-ion concentration.

$$pH = -\log[H^+], \quad \text{or} \quad pH = \log\frac{1}{[H^+]}$$

It is slightly confusing that decreasing acidity (10^{-5}, 10^{-6}, 10^{-7}, etc.) should mean increasing *p*H (5, 6, 7, etc.) but the mind soon becomes adjusted to this apparent paradox.

It is important also to remember that whereas the difference between two *p*H values such as 2, that of 0.01 *N* HCl or average lemon juice, and 7, that of pure water, is 5, this difference expressed in terms of the actual concentration of hydrogen ions is 100,000, i. e., *p*H 2 has 100,000 (10 × 10 × 10 × 10 × 10) times as many H^+ ions as has *p*H 7.

Some idea of the range in *p*H values is given by the following table.

NORMALITY	*p*H	H^+ MOLES/L	OH^- MOLES/L
N HCl	0.0	1	10^{-14}
0.1 HCl	1.0	10^{-1}	10^{-13}
0.01 HCl	2.0	10^{-2}	10^{-12}
0.001 HCl	3.0	10^{-3}	10^{-11}
0.0001 HCl	4.0	10^{-4}	10^{-10}
0.00001 HCl	5.0	10^{-5}	10^{-9}
0.000001 HCl	6.0	10^{-6}	10^{-8}
Neutrality	7.0	10^{-7}	10^{-7}
0.000001 NaOH	8.0	10^{-8}	10^{-6}
0.00001 NaOH	9.0	10^{-9}	10^{-5}
0.0001 NaOH	10.0	10^{-10}	10^{-4}
0.001 NaOH	11.0	10^{-11}	10^{-3}
0.01 NaOH	12.0	10^{-12}	10^{-2}
0.1 NaOH	13.0	10^{-13}	10^{-1}
N NaOH	14.0	10^{-14}	1

BUFFERS

If a certain quantity of hydrochloric acid is added to water, the *p*H is lowered correspondingly, but if the same quantity of acid is added to a beef infusion, the change in *p*H will be very slight. The power of certain solutions to resist to some extent a sudden change in acidity was likened to a tampon, the old French of which is *buffe*. A tampon is a swab or sponge which soaks up excess fluid; just so does a *p*H buffer "soak" up excess hydrogen ions and yield them when there is a deficiency. A tampon in German is "Puffer," which became "buffer" in English, not a good translation. Most biological fluids, such as plant juices, blood, and protoplasm are well buffered. Buffers convert strong acids into weak ones; thus, the strong acid HCl added to the buffer $NaHCO_3$ results in the weak acid H_2CO_3 and the neutral salt NaCl.

Buffers also function in stabilizing alkalinity. This is obvious from the fact that buffers always involve equilibria; they maintain a balance.

COLOR DETERMINATIONS OF *p*H

The commonplace test for acidity is taste. Color change in such liquids as wine and "syrup of violets" was early known to indicate acidity. The animal pigment "cochineal" extracted from the insect of that name and the plant pigment, "litmus," from lichens, have long been used to indicate acid and alkaline conditions. Litmus is still used as a common acid-alkaline indicator. Coal-tar dyes once discovered soon took the place of plant and animal pigments as *p*H indicators.

Sørensen calibrated the color changes that certain coal-tar dyes undergo with change in acidity, and these now serve for making *p*H measurements. A comparison is made of the colors assumed by the dyes when added to solutions of known and of unknown *p*H values. This is most easily accomplished by placing drops of the unknown and the known solutions on a white porcelain plate and then adding the indicator. Comparison with standard tubes of prepared dyes may also be made. Different hues of color in nature often result from the same pigment with change in acidity: thus, anthocyanin is responsible for both the blue of the cornflower (at *p*H 7.2) and the red of the rose (at *p*H 5.5). Purple cabbage juice gives an excellent color range when small and increasing amounts of acid and alkali are added, the color changing from a bright red at *p*H 1, through lilac at *p*H 3, pur-

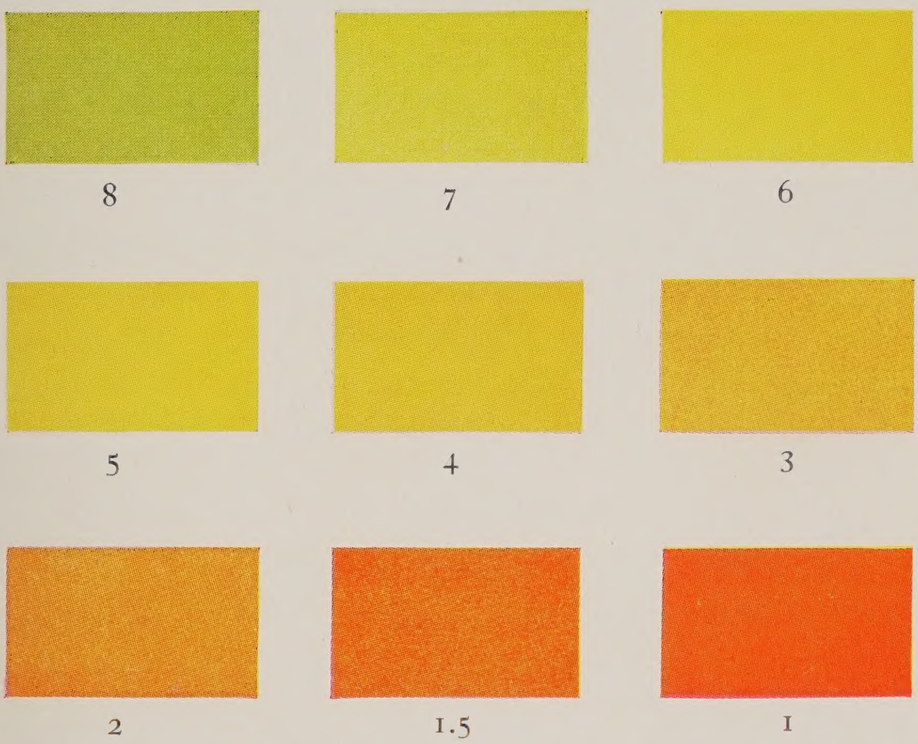

Fig. 33. A *p*H chart: These colors are assumed by the yellow pigment of the slime mold, *Physarum polycephalum,* at the indicated acidity values from *p*H 1 to *p*H 8; *p*H 2 to 7 occur in the living plant; 6 is the *p*H value and color of the young and active plasmodium.

ple at pH 5, blue at pH 6, to green at pH 8. The yellow pigment of some slime molds is also a good pH indicator, the range being from deep red-orange at pH 2 through yellow to green at pH 8 (Fig. 33).

POTENTIOMETRIC DETERMINATIONS OF *p*H

If two solutions of different ionic concentration are electrically connected, the difference in potential between them can be measured; and, if the potential of one electrode is known, the other is readily calculated. To measure these potentials when they are weak, a *potentiometer* is used. With its help, two electrodes, one of known potential, called the reference electrode, and one of unknown potential, are brought into equilibrium. If the potential of the unknown electrode is produced by hydrogen ions, then it must be a measure of the hydrogen-ion concentration, or activity. It now remains only to convert the potential produced by the hydrogen ions into pH. This is accomplished by the use of a formula or table.

THEORY

Barely had the classical work of Sørensen on hydrogen-ion concentration become familiar to chemical and biological workers than doubt was cast on the validity of his assumption that $p\text{H} = \log \frac{1}{[\text{H}^+]}$. Values obtained with other solutions did not agree with his findings. The difficulty was not known, but a correction factor was obviously necessary. Possibly the methods of measurement—colorimetric, electrometric, or kinetic—were responsible, yet all, properly handled, gave results which agreed very closely. Apparently some unknown property of the solution was causing its anomalous behavior.

Twenty-five years after Arrhenius' contribution, Bjerrum advanced the theory of *complete dissociation*, which states that strong electrolytes are always fully separated into their ions even at high concentration. He asserted that decrease in electrical conductivity and in depression of the freezing point that accompanies increase in concentration, which led to the belief that electrolytes are but partially dissociated, was due not to incomplete dissociation but to the influence of the electric charges of the ions on each other. The molecular conductivity is diminished not because of a smaller number of ions, but because ionic movements are retarded. This is accomplished by the electrical atmospheres of the ions which hinder each other's progress. From this hypothesis arose the concept of *activity*.

CONCENTRATION VS. ACTIVITY

Acidity in terms of hydrogen ions, or pH, is usually defined and expressed as a concentration. When Sørensen recommended the symbol $p\mathrm{H}^+$, he thought he was measuring the hydrogen-ion concentration, which he expressed in terms of the negative logarithm: $p\mathrm{H} = -\log[\mathrm{H}^+]$. That concentration is a factor is shown by the different pH values which result when the concentration of an acid or alkali is changed. Although it is still convenient to express pH as a quantity factor, it is *activity* which is measured, colorimetrically or electrometrically; therefore, instead of pH being an expression of concentration, it is really an expression of activity: $p\mathrm{H} = -\log a\mathrm{H}^+$. This may at first be confusing, but it is possible to arrive at a definition of activity in terms of concentration through the equation:

$$a = Cf$$

where a is the activity, C the concentration, and f the activity coefficient. The coefficient f varies for different ions and different concentrations; it may be determined experimentally or by calculation.

The definition of pH based upon concentration may be maintained, for it represents the negative logarithm of a quantity, and in many cases a knowledge of the concentration of the hydrogen ion is more important than a knowledge of its activity. The numerical difference between activity and concentration, in moderately dilute solution, is moreover not very great.

BIOLOGICAL APPLICATIONS

The availability of nutrients to the plant is to a great extent determined by the pH of the soil. Certain plants, such as rhododendron and members of the heath family generally, are known as acid-soil plants. Sugar beets have their highest yield in sugar when grown on neutral soils. Soils vary in the constancy with which they hold their *p*H value, some being fairly well buffered with aluminum silicate complexes (clay) and organic colloids.

Marine plants are often closely adjusted to the acidity of the ocean, the *p*H of which averages 8.2. The water near coral reefs is more alkaline than the water off rocky shores, and a marine plant growing in the one region will not necessarily grow in the other.

Chlorosis, a disease indicated by lack of green color in leaves, when not due to deficiency of magnesium, manganese, etc., presents a problem in acidity. Chlorosis is a symptom, a generic name for what was at first thought to be simply iron deficiency but now appears to be a whole range of disorders. Adding iron to chlorotic plants usually cures the disease, but in certain cases there is sufficient iron in both the soil and the plant. Other factors, therefore, must be involved in such cases. One of these is alkalinity; therefore, acid, not iron, should be added. Pear trees of the Crimea are often so badly chlorotic that one wonders how they can continue to live and produce fruit. There is sufficient iron present, but the plants cannot make use of it because of a highly alkaline soil. The addition of acid reduces alkalinity and puts the trees in a condition which enables them to use the available iron. Although this is true, the story is not so simple a one. Solubility of the iron will depend not only on acidity but also on the form in which the iron occurs. Iron salts of inorganic and simple organic acids are more readily soluble in acid than in alkaline solutions; but iron salts of complex organic compounds are more soluble in an alkaline than in an acid medium. Consequently, alkali-induced chlorosis does not exist on soils rich in humus.

Some experimental data found by Shive would seem to contradict the foregoing conclusions, but closer investigation reveals the true situation. Shive found that iron accumulations occurred in plants with high *p*H (alkaline), whereas more acid plants had a low iron content; but in the relatively alkaline plants iron was found to be mostly precipitated and therefore not available, whereas the iron in the acid plants though small in quantity was uniformly distributed and all available.

Most crop plants require a slightly acid soil. When acidity becomes too high, lime should be added. However, a soil which the farmer calls sour is often only slightly acid, if at all so; it is primarily simply poor soil, poor in essential nutrient elements. The cure in such cases is not an alkali but a fertilizer.

For acidifying soils, peat moss, now imported in large quantities from the peat lands of northern Europe, is most satisfactory and inexpensive. It adds also to the friability and water-holding power of the soil. Oak leaves may also be used, or the residues of bark from tanneries, which have a fairly high concentration of tannic acid. The most suitable and inexpensive of salts for acidifying soil is aluminum sulphate, to be had at waterworks where it is used to precipitate colloidal matter.

The sensitivity of certain plants to hydrogen-ion concentration is sufficient proof of the importance of acidity in physiology and agriculture; yet it is also true that some plants show extraordinary tolerance. Alfalfa will give 100 per cent germination in a soil with a *p*H as low as 4.

The *p*H of a soil varies with geological formation, with depth, and with the flora. Acidity to a great extent determines the type of plant life, but, once the vegetation is well established, it may, in turn, have a pronounced influence on the *p*H of the soil. Farm lands average about *p*H 6, maple-beech forest 5.5, and pine or oak forest 4.5. The *p*H of most soils increases with depth, from a *p*H of 5 at the surface to *p*H 6 or more several feet below. Greater acidity at the surface is due to the leaching of the soil by rain as it dissolves the alkaline matter at the surface and carries it to lower levels.

The role of *p*H is important throughout nature. Plant and animal distribution, the development of organisms, and their well-being are all determined or influenced by acidity. Whitaker gives an illustration of the part played by acidity in the development of the eggs of the seaweed *Fucus*.

Normal sea water has a *p*H of 8.2. The eggs of *Fucus furcatus* develop perfectly in sea water acidified to *p*H 6.0; development is retarded at *p*H 5.5 and stopped at *p*H 5.0. Further, there is a mutual inductive effect when there are numerous eggs in the dish. The eggs develop rhizoids toward each other when there are several in a dish; the peripheral eggs, having no neighbors on the outer side, develop their rhizoids in the direction of their neighbors in the center. The polarity and the whole developmental pattern of the embryo are thereby induced. This inductive effect does not operate in normal sea water when only two eggs are present; but, if the sea water is acidified to *p*H 6, two eggs alone in a dish will develop rhizoids on the sides toward each other. Increased hydrogen-ion concentration thus augments or intensifies the mutual inductive effect. The induction may be due to a hormone the activity of which is intensified by a lowered *p*H.

Possibly undue importance has been attributed to acidity in some instances, yet it is still true that in physiological reactions, as in many non-living chemical ones, the H^+ ion is the most significant of all ions.

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CHAPTER X

SALT REQUIREMENTS

Inorganic salts are necessary to life. They supply the elements required by plants and animals to build material, establish osmotic and electrical forces, catalyze reactions, and organize activities. The fact that certain elements are necessary for plant life may be ascertained in a number of ways. There is, first, the knowledge that plants require water and air, from which it may be assumed that hydrogen and oxygen are needed. The farming experience of generations has shown that plants thrive better and yield larger crops if nitrates, phosphates, and lime are added to the soil, from which it may be deduced that nitrogen, phosphorus, calcium, and possibly also potassium are used by the plant. As the plant is known to synthesize organic compounds, carbon must, by one route or another, find its way into the plant. Unless it is shown that plants can use organic food absorbed from the soil, it is a safe assumption that carbon in the form of carbon dioxide is obtained from the air. Experimentation shows this to be true.

The earliest experimenters on plant nutrition failed to make the deduction that any essential element was supplied by the air. The experiment of van Helmont, cited in the Introduction (page 7), led that investigator to assume that all the weight gained by the plant must have come from the water added, since the soil lost very little in weight. He failed to take into account the air, which is now known to be the sole source of carbon for the plant. A knowledge of plant products leads to the conclusion that the plant's elemental needs include carbon, hydrogen, oxygen, nitrogen, phosphorus, calcium, and potassium. It is only necessary to add sulphur, magnesium, and iron to complete the primary list of ten essential elements for plant nutrition. This list long stood as the complete requirement for plant life, but gradually, with the development of improved technique for investigation, other elements took their places among the essential ones, until the list is now well over twenty.

A chemical analysis of the plant should indicate its nutritional needs. This is true in the main, but certain of the elements found may be tolerated only and not needed by the plant.

The growing of plants in soils or solutions of known composition is the usual experimental method of ascertaining the salt requirements of a plant. From each sample of soil or solution, one element is eliminated and subsequent growth of the plant noted. Soil is not a satisfactory substratum for such experiments because of the difficulty of ascertaining its exact composition. Even the purest quartz sand requires much washing before one can be only reasonably certain that it contains no foreign elements. More satisfactory is the method known as water-culturing.

WATER CULTURE

The search for greater knowledge of the roles played by the essential elements in plant nutrition led to the technique known as *water culture.* Culture solutions for the growing of plants were developed by Sachs and Knop about 1860 and later improved by Pfeffer. At the same time, Ringer was studying the effects of salts on extirpated animal tissues. He found that an isolated heart would continue to beat if perfused with saline solution. Beating was prolonged by the addition of calcium to the sodium chloride and still more so by adding potassium. Thus evolved the now renowned *Ringer's solution*, a physiologically balanced solution suitable for bathing animal tissues so as to keep them alive when isolated. There is a fundamental similarity between the salt constitution of Ringer's solution and that of sea water, milk, and blood.

The study of the salts essential to plant life has developed along lines similar to those followed in animal physiology. What Ringer's solution has been for animal organ and tissue culture, the solutions of Knop, Pfeffer, and Shive have been for plant cultures. Careful study of the needs of plants when grown in culture solutions led to the development of a three-salt nutrient solution by Shive. The best-known culture solutions are listed below, with the partial salt concentration of the ocean added for comparison (sea water contains traces of other elements, e.g., bromides and iodides).

Salts	Sachs	Knop	Pfeffer	Shive	Ringer	Ocean
KCl	...	0.12	0.1	...	0.14	0.58
NaCl	0.5	...	...	...	6.5	26.86
KNO_3	1.0	...	0.2	...	...	...
$MgCl_2$	...	1.0	0.8	...	...	3.24
$Ca(NO_3)_2$	...	...	...	0.85	...	...
$CaCl_2$	...	...	...	...	0.12	...
$MgSO_4$	0.5	0.25	0.2	1.8	...	2.20
$CaSO_4$	0.5	...	...	...	...	1.35
KH_2PO_4	...	0.25	0.2	2.5	...	...
NaH_2PO_4	...	...	...	...	0.01	...
$CaPO_4$	0.5	...	...	...	...	...
$NaHCO_3$	...	...	...	...	0.2	...
$FeCl_3$	trace	trace	trace	...	...	...

Later research having revealed the importance of boron and manganese to plant life, Shive now recommends adding a trace of soluble boron (as H_3BO_3) and of manganese ($MnSO_4$) to his three-salt solution (see page 110).

Experiments with water cultures demonstrate in a most striking way which elements are necessary for plant life (Fig. 34). Standard nutrient solutions serve as controls. Carbon, hydrogen, and oxygen come from water and the air, and they cannot, therefore, be studied by the elimination method. The plant's need for them, however, is readily demonstrated in other ways.

The foregoing nutrient solutions were tested by Philip R. White and compared with a standard solution developed by him—it is essentially a Pfeffer solution with reduced phosphate content, having a salt concentration of 2.7 millimols. Keeping the salt proportions of all the nutrient solutions unchanged and using excised tomato roots as test objects (page 240), White found his solution to give the best results, with Shive's three-salt solution as next best. The White solution contains 0.6 millimols of $Ca(NO_3)_2$, 0.9 KCl, 0.8 of KNO_3, 0.1 KH_2PO_4, 0.3 $MgSO_4$, and 0.006 $Fe_2(SO_4)_3$. It is possible that this solution may surpass others in the growth of whole plants in culture.

White further demonstrated that the suitability of a number of solutions as nutrients for isolated roots could be considerably improved by increasing their acidity. He found all satisfactory solutions to have *p*H values below 6.

Raines has developed a wick culture technique by means of which a plant may be grown with its root system confined to the thin space between wet black blotting paper and a sheet of glass. The culture solution moves through the paper at a rate that may be regulated. The method permits ready observation of the root system throughout the experiment.

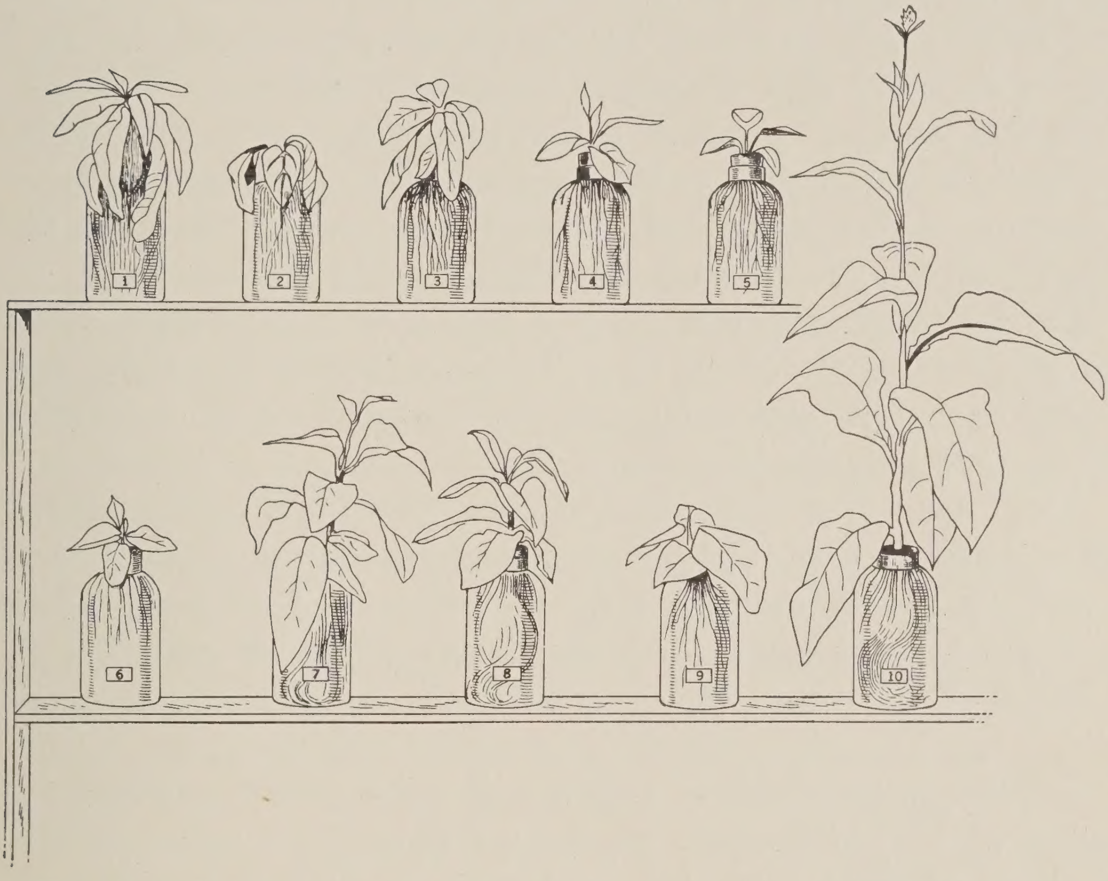

FIG. 34. Tobacco plants in culture solutions from which one of the ten necessary elements is omitted; the element omitted in solution 1 is magnesium, 2 calcium, 3 potassium, 4 phosphorus, 5 nitrogen, 6 iron, 7 manganese, 8 sulphur, 9 boron, in 10 all of the necessary elements are included.

THE PRIMARY ESSENTIAL ELEMENTS

Elements essential to life may be classed as *nutritive* or *regulatory*. Carbon, hydrogen, oxygen, and nitrogen are nutritive. Potassium and calcium are primarily regulatory. Sulphur, and possibly also phosphorus, fall into both categories; they serve not only as tissue builders, as constituents of proteins and protoplasm, but also as regulators of reactions.

The regulatory role of an element may be purely physicochemical, for example, as catalyst, or it may involve complex biological processes which cannot be stated specifically. Culture solutions must satisfy these two requirements if the best growth is to result; they must contain all the essential elements and must be physiologically balanced, that is to say, they must be non-toxic and nutritive. It is, therefore, necessary to recognize the dual nature of every element, physiologically considered. All conditions and materials essential to life influence growth, and, when an element is described as accelerating root formation, the only deduction that can be made is that it is *one* of the elements influencing the growth of the *particular* tissue studied.

Carbon, hydrogen, and oxygen, play their chief part in plant life as constituents of foods, cellulose, accessory products, and protoplasm. Nitrogen enters into the composition of the higher foods, especially the proteins.

Magnesium is a constituent of chlorophyll and as such establishes itself as one of the most important of the essential elements of plants, though the amount needed is small. The presence of magnesium in plant tissues which do not contain chlorophyll suggests a regulatory function as well as a nutritive one.

Sulphur, as a nutritive element, is an essential part of the amino acid, *cystine*. As a regulatory element, it influences the synthesis of chlorophyll and accelerates root formation.

Phosphorus is a frequent constituent of proteins, in particular, the nucleoproteins. It is also necessary for the formation of lecithin and nucleic acid, which are present in all living plant cells.

Iron is a very important element for both plants and animals, its function in the two cases being somewhat different. In animals, iron is a constituent of hemoglobin, where it serves as a carrier of oxygen. In plants, iron is not a constituent of the corresponding plant pigment, chlorophyll, but serves as a catalyst or activator in the synthesis of chlorophyll. Plants which cannot obtain sufficient iron from the salts of the soil are unable to produce chlorophyll; they are yellow in color and are said to be *chlorotic*. Where chlorosis is a simple case of iron deficiency iron may be added directly. Chlorosis of citrus trees is overcome by putting iron citrate into holes bored into the trees (see also page 99).

Potassium and calcium are necessary for plant life, but the roles which they play are unknown. Potassium has long been recognized as one of the three principal constituents of fertilizers, nitrogen and phosphorus being the other two. There is no conclusive evidence that potassium enters into the formation of the body material in plants, that is to say, it is not a tissue builder. Its role appears to be wholly catalytic. Its occurrence in the plant chiefly as an inorganic salt, readily soluble in water, is further evidence of its purely formative or regulatory function. Certain foods cannot be synthesized without the aid of potassium. Starch formation does not progress without this element; and the synthesis of proteins and fats is augmented by it. Potassium increases the diastatic activity, i.e., the activity of enzyme action in the breakdown of starch.

The last of the foregoing reactions is not a direct one, i.e., potassium does not accelerate diastatic activity, when added to the extracted enzyme. This illustrates an important truth in all nutritional work, namely, that whereas a substance may be directly correlated with a specific pathological disturbance, yet, as any upset in the normal metabolism of an organism will lead to an abnormal condition, then one must be exceedingly cautious in attributing a direct effect to one particular element. Potassium is essential to the life of every cell; its absence, therefore, will mean a disturbance of a number of physiological processes. It has been found that starch formation and starch digestion do not progress without potassium, although this is true only in living tissue. Consequently, the conclusion that potassium affects starch formation and starch digestion is justified only in so far as a disturbance in starch metabolism has served as the indicator of a general disturbance in catabolic and anabolic activities.

The roles played by calcium and potassium are similar, except that calcium possibly enters into the building of the skeletal framework of plants, just as it does in animals, where it helps to build bone. Calcium may be utilized in the formation of the cellulose walls of plant cells. The middle lamella has long been thought to consist of calcium pectate. If this is true then calcium is needed for its formation. But the needs of calcium to the plant are greater than for the building of walls. It may have an influence on the translocation of carbohydrates, and possibly to an extent determines the physiological availability of other ions. Schimper in 1888 suggested that one of the functions of calcium is to precipitate oxalic acid and soluble oxalates in the form of calcium oxalate, thus preventing the injurious effects which they might have upon the plant.

The foregoing ten elements are those upon which, for many years, emphasis has been laid as the essential elements of plant nutrition. Several others have been considered as possibly beneficial to plants because of their occurrence in certain species and their abundance in soils; thus sodium and chlorine are among the most common components of soil salts; silicon is a constant constituent of many plants, and occurs in large amounts in most grasses; and iodine is commercially obtained from brown seaweeds, which concentrate it from the sea water in which they grow.

Sodium is often cited as a distinguishing feature of the salt needs of plants and animals; plants are presumed not to need it, whereas animals unquestionably do. However, as sodium occurs in fairly large amounts in some plants, it may play a role there, though probably not one of great consequence. Observation certainly indicates that some plants are benefited by its presence in the soil. As sodium makes up three-fourths of the total salt content of the ocean, it would appear likely that marine plants require it. If this is true, its significance is unknown.

Chlorine is an element of extraordinarily wide distribution in the living as well as the non-living world, yet it seems to be relatively inconsequential to life except in an isolated though important instance in the animal kingdom, namely, as part of the hydrochloric acid in gastric juice. For plants, chlorine does not appear to be beneficial, although the literature on the subject leaves the whole question in doubt. In high concentration it is certainly harmful.

Silicon is another element concerning which much has been said both for and against its being an essential component of plant tissue. That it, as silica or sand, may add stiffness to grasses and other plants, e.g., to the scouring rush, *Equisetum*, which possess it in considerable quantities, was early suspected, but the evidence is unconvincing. The suggestion has also been made that plants are more resistant to disease when containing silicon.

Next to silicon, aluminum is the most abundant metallic element in the soil. In combination with oxygen, it forms alumina (Al_2O_3) which enters into the composition of a large number of minerals, the most important of which are feldspars. The decomposition of these yield clay, a hydrated silicate of aluminum. Aluminum is found in plant ashes and may possibly be needed by plants.

Before turning to other elements necessary for plant life, the position in the periodic table of the fourteen so far mentioned will be worth noting.

	I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*
1.	H	—	—	—	—	—	—	He
2.	Li	Be	B	C	N	O	F	Ne
3.	Na	Mg	Al	Si	P	S	Cl	A
4.	K	Ca	Sc	Ti	V	Cr	Mn	Fe
5.	Cu	Zn	Ga					

Iodine is a common constituent of seaweeds. Indeed brown algae are the chief commercial source of iodine. Bromine and fluorine are likewise more or less concentrated by seaweeds. Again the question arises; is a salt necessary to a plant because it is a common constituent of that plant? The presence of iodine may be a case of tolerance. Experiments on terrestrial plants, some of which contain appreciable amounts of iodine, fail to reveal any benefit to the plant from this element, but this does not bear directly on the situation with respect to marine plants. The "benefit" of an element may be an indirect one; that is to say, as all previous generations of a plant have been exposed to an element, they may have developed mechanisms to take care of it, and now, if the element is absent, the mechanisms might act upon other substances so as to produce harm.

P. R. White finds the following elements essential for roots in culture (page 241) : carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, calcium, potassium, manganese, zinc, boron, iron, and iodine. Chlorine and magnesium are possibly necessary, but the evidence is not conclusive. Copper may also be needed, but proof of the need is weak. If any other elements are essential for roots in culture, they must be so only in extraordinarily low concentrations, less than 10^{-10}.

TRACE ELEMENTS

The quantity of iron required by a plant is very slight. It is, therefore, known as a trace element. Its need to the plant was early recognized, and so it became one of the first ten essential elements. Its significance has been discussed above.

Manganese is a trace element the absolute need of which by plant life is now established beyond all doubt, but too many functions have been attributed to it to permit regarding any one of them as having been definitely established. It can only be said that manganese is necessary to the normal metabolic processes of a plant, possibly in protein synthesis and the production of chlorophyll. The dilution of manganese which suffices for the aquatic plant, *Lemna major*, is the almost incredible one of 1 :3,000,000,000.

Boron is an unexpected element to be suddenly raised to a position of prime importance in plant life. Like manganese, it is absolutely necessary for normal growth but its precise function is unknown, that of a catalyst or regulator being the most likely. The role of boron as an activator of physiological processes is illustrated in an experiment by Schmucker who, finding that the pollen of the water lily would not germinate in the artificial medium which he had prepared, analyzed the sticky substance on the pistil and found it to contain boron. On adding this element to the artificial medium, the pollen germinated. Whether termed catalyst or "inorganic hormone" the simple fact remains that boron activates a growth process (see page 213). Though of wide distribution, it occurs only in exceedingly minute quantities in plants.

Copper finds a probable need in plant nutrition in physiological association with iron, as it does in animals.

Zinc is known to give beneficial results in certain cases when added to the soil. Zinc deficiency is the specific cause of "mottle leaf" in citrus trees.

The total number of elements now known to occur in plants is forty-three. The following list includes all so far reported, in approximate order of apparent importance:

Carbon
Hydrogen
Oxygen
Nitrogen
Potassium
Calcium
Phosphorus
Magnesium
Sulphur
Iron
Sodium
Chlorine
Iodine
Bromine
Fluorine
Silicon
Boron
Lithium
Barium
Strontium
Manganese
Copper
Zinc
Tin
Aluminum
Rubidium
Cobalt
Lead
Nickel
Mercury
Arsenic
Selenium
Thorium
Thallium
Titanium
Silver
Gold
Vanadium
Radium
Uranium
Chromium
Scandium
Beryllium

A number of interesting questions arise in regard to the trace elements. One wonders why their presence was so long overlooked, and how it is that plants seem to grow well in culture solutions to which only the ten essential elements first enumerated have been added. There is also the question of substitution; is this or that particular element necessary, or will another do as well?

Failure to detect the trace elements in plants was due to inaccurate methods of analysis. The quantities are too small to permit determination by simple analytical methods, for the plant deals with concentrations far below those with which the chemist is ordinarily concerned. Not until the detection of rare elements in the form of incandescent gas was made possible through development of the spectroscope was the botanist able to determine their presence in plants.

The good growth of plants frequently observed in culture solutions to which the trace elements have not been added is to be explained by the fact that few commercial salts, such as are commonly used in making up these solutions, are free of foreign elements; the trace elements are, therefore, not absent.

Sometimes, even though every precaution is taken to eliminate all "impurities" in the culture-solutions, the plants may still appear to be healthy. This may simply mean that weaknesses in the plant are hidden; under the rigors of outdoor life and competition with other plants they would appear and the plants succumb.

CORRELATIONS

An important fact to be kept in mind when considering the nutritional needs of plants is the correlation interrelationship of the essential elements. An element may have no direct bearing on a physiological process, but its presence may be necessary in order that some other element may play its role. There is a close relationship between potassium and nitrogen. The environment also plays a part in determining the functioning of salts: thus light, or length of day, determines to a great extent the nutritional value of numerous elements.

The chlorotic condition of plants is the symptom of a general disturbance, and involves the interrelationship between calcium, magnesium, and iron. There is in certain parts of New England a chlorotic condition of plants that is cured not by the addition of iron, but of magnesium. Farmers there use only dolomitic lime or lime with magnesium added. The function of the magnesium is in this instance to maintain a balance between elements for the prevention of chlorosis.

Chlorotic plants often have sufficient iron present but cannot make use of it. There are a number of possible reasons: magnesium deficiency may be responsible; the acid-alkaline condition of the soil or plant may not be suitable (page 99); or the iron may not be in a form suitable for use by the plant; it may, for example, be bound to an organic compound, to the so-called humic acids of soils, while the plant requires it as a salt.

The balance between magnesium and other elements affects numerous conditions. Some plants do not have a very good mechanism for taking care of excess calcium; magnesium is involved here. Magnesium increases the transportation of the phosphate ion, so that too much calcium and too little magnesium result in too little phosphate. The phosphate story is further complicated by the low solubility of iron phosphate, which is the chief cause of the unavailability of phosphate in the soils of the northern United States. There are thus four elements, phosphate, iron, calcium, and magnesium, concerned in an interrelationship involving the availability of but one of them.

No element plays its role alone; its relation to other elements and the environment determine, in part, its role in plant life.

TOXICITY

Any salt is toxic if its concentration is high enough. In the case of trace elements the concentration need not be very great to produce highly injurious effects. This is well illustrated in the now classical example of copper. Naegeli found that water, in which a few copper coins had remained for a time, quickly killed the filamentous alga, *Spirogyra.* Dilution of the copper water until by calculation but one molecule of copper remained in a liter of water left it still poisonous. Naegeli rinsed the glass thoroughly and substituted fresh distilled water, but still the alga died. The effect was so startling that he gave to it the distinguishing name, *oligodynamic.* Undoubtedly more copper ions were present than Naegeli thought, probably adsorbed to the glass, but the experiment at least showed copper to be very toxic in low concentrations.

Silver and other heavy metals are equally poisonous. A silver coin laid in a culture of bacteria on agar will result in a space left free from bacteria around the coin owing to minute quantities of the metal which have gone into solution.

Naegeli's experiment on copper proved to be of commercial value. On one occasion, water-cress growers found their crop dying from some unknown cause, which on investigation proved to be smothering by *Spirogyra.* The investigator in charge, recalling Naegeli's experiment, added copper in the concentration of 1:50,000,000, which destroyed the *Spirogyra*, and left the cress unharmed. In a similar way, mosquito larvae in stagnant water and pathogenic bacteria in drinking water may be killed.

The manner in which organisms handle large concentrations of toxic elements is not known, but they often store surprisingly great quantities. The feather of the red-crested touracou is said to contain as much as 15 per cent copper. Plants are often able to handle certain elements without apparent ill effect, yet the plants when eaten are very toxic to man and animals. Selenium is a startling case of recent discovery. It holds the unique, but none too honorable, position of being the only element absorbed from virgin soil which causes disease. The "alkali disease" of cattle is due to eating plants containing selenium. Alfalfa grown on one Mexican ranch was reported to be the cause of the loss of 125 head of cattle in a night's feeding. Indians are made ill by it as well as cattle. Animals thus suffer severely from an element which plants tolerate.

It is not only the heavy metals which are toxic. The common essential elements are so as well. Their toxicity constitutes a special problem which is known as *salt antagonism.*

ANTAGONISM

A plant requires certain salts for its complete nutrition; if any are lacking, the plant suffers. Obviously, a salt or a condition which is lacking can do no harm. Though a plant needs potassium, the potassium which is absent cannot injure it. The injury must, therefore, result from those elements which are present; but as these elements are needed they should be beneficial. So they are, but only in the presence of certain others and in proper proportions. In short, when all essential elements are present in proper proportions, a solution is non-toxic; it is then known as a *normal* or *physiologically balanced* solution. The elements necessary to plant life are toxic when alone. This toxicity is counteracted by other elements when all are together. The effect which elements have upon one another in a physiologically balanced solution which renders each of them non-toxic is known as *salt antagonism.*

The term "antagonism" applied to the balancing or antitoxic effect of elements is an unfortunate one. It implies that the two elements which "antagonize" each other are quarrelsome enemies and "have it out" between themselves, thus leaving the plant in peace. Some such idea may have been in the minds of the earlier workers.

The first recorded case of antagonism is that discovered by

Ringer. He found that calcium chloride added to sodium chloride decreases the harmful effect of the latter on animal tissues. Much work has been done on antagonism, but there is little to show for it except a few generalities. The rule that monovalent cations (Na) antagonize bivalent ones (Ca) holds in a broad way, but by no means in all cases; for example, the toxic effect of both sodium sulphate and magnesium sulphate on corn and lupines is lessened by the addition of calcium sulphate. Calcium also antagonizes barium. Thus does a bivalent element decrease the harmful effects of both a monovalent and a bivalent element. The matter is further complicated by an antagonistic action between cations and anions.

Magnesium, though necessary to plant growth, may, when certain other elements are not present, produce magnesium injury. Tottingham and Trelease have demonstrated how conspicuous and distinctive are the symptoms of magnesium injury, and Trelease has shown that calcium in sufficient quantity will prevent magnesium injury. The action of the calcium is an example of salt antagonism, and illustrates further that one bivalent cation may antagonize another.

The mechanism of antagonism is not known. It may, as suggested, take place between the ions themselves before they enter a cell; but this seems unlikely. More reasonable is the suggestion that ions which exhibit antagonistic action exercise an opposite effect on protoplasm. There is substantial experimental evidence in support of this in the case of sodium and calcium. Sodium lowers protoplasmic viscosity and calcium raises it, that is to say, sodium disperses and calcium aggregates. This fact is supported by the effects of sodium and calcium on protoplasmic elasticity, permeability, and coagulability. Protoplasm is poorly elastic in the presence of sodium, and highly so with calcium. Sodium increases and calcium decreases protoplasmic permeability. Calcium augments wound-healing; a torn protoplasmic surface is repaired in the presence of calcium, but not in the presence of sodium, owing to the coagulating effect of the former ion. It thus appears probable that the antagonistic action of sodium and calcium is due to opposite effects of these ions on protoplasm, to the dispersing or liquefying effect of sodium and the aggregating or coagulating effect of calcium.

The proportion of sodium to calcium which establishes perfect antagonism is of significance because it is the same proportion at which these elements occur in sea water, blood, milk, and other biological fluids; namely, fifty parts of sodium to one of calcium.

LYOTROPIC SERIES

The different effects of ions on protoplasm and proteins gave rise to the *Hofmeister* or *lyotropic series.* The original series discovered by Hofmeister was based on the relative coagulating effects of certain anions on albumin; the ions were found to be effective in the following order: citrate>tartrate>sulphate>acetate>chloride>nitrate>chlorate, citrate causing the greatest and most rapid coagulation, and chlorate the least. It was later maintained that these differences are not specific but are due solely to valence. Furthermore, differences within a valence group were denied except in so far as these were due to differences in acidity. If acidity is kept constant, the series ceases to exist except in so far as the ions fall into valence groups. Subsequent research proved that this contention did not hold; however, those who advanced it were justified in calling attention to the important effect of acidity. For example, aluminum increases acidity; therefore, much of the effect of this ion on protoplasm is due to change in acidity, the rest (about one-half) is due to the aluminum ion.

If certain cations are grouped on the basis of their effects on the elastic qualities of protoplasm, they fall into the following order:

$$\mathrm{Ca} > \mathrm{Sr} > \mathrm{Mg} > \mathrm{K} > \mathrm{Li} > \mathrm{Na}$$

Acidity does not determine this grouping, for the chlorides of these elements produce little or no change in acidity. Although the ions fall into two groups, monovalent and bivalent ones, yet there are pronounced differences between the members of each group. Calcium greatly increases the stretching capacity of protoplasm; magnesium has no effect. Potassium lowers the elasticity but slightly; sodium almost wholly eliminates it.

SUBSTITUTION

Similarities in the properties of elements have been the basis of their chemical classification. The periodic table illustrates this. The colloid chemist would predict that the members of any one group, such as sodium, potassium, and lithium, or calcium, barium, and strontium, will produce similar effects upon a property, say swelling, of such a system as gelatine. But the biologist knows that in spite of chemical similarities, the elements are not identical and chemical differences make themselves apparent in physiological reactions. For example, all plants need potassium and few if any need sodium; calcium is an important element in plant and animal life whereas barium in anything like the same concentration is highly poisonous. It is not possible, therefore, to supplant potassium by sodium, or calcium by barium, in the salt needs of organisms. In spite of these facts, the hypothesis gained ground that one element would replace another of the same valence in regard to their purely physical roles, such as the capacity to antagonize. Only to a limited degree is this true.

Elements of the same valence will not always replace each other in their antagonistic action; thus, magnesium will not replace calcium. Still less likely is it that one element can be substituted for another in a nutritive capacity. The plant demands potassium, and sodium will not do as a substitute. McHargue finds that no other one of the more common elements, including iron, copper, zinc, or boron, will replace manganese in the growth of plants, and Brenchley finds that no element can take the place of boron.

ADJUSTMENT TO CHANGES IN SALTS

Organisms are usually nicely adjusted to their salt environment, and are very sensitive to a change in it, but occasionally they adapt themselves to sudden and pronounced change in salt concentration with surprising ease. Plants and animals which live in the estuaries of rivers must tolerate fresh water at ebb tide and salt water with the incoming tide. In animals, change in the salt environment more often brings on abnormal development. Plants, however, must withstand a considerable range, for the salt concentration of the soil is changing all the time.

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CHAPTER XI

PERMEABILITY AND THE PROTOPLASMIC MEMBRANE

The marine alga, *Valonia* (Fig. 35), stores within its large central vacuole forty times as much potassium as sodium. Whereas the concentration of potassium within the vacuole is many times that in sea water, the concentration of sodium is only one-fifth as great as in the surrounding water. *Valonia,* therefore, has the power to select certain specific salts from among the substances which surround it. The capacity of cells to select is presumed to rest upon properties peculiar to the surface layer, or protoplasmic membrane. Protoplasm is moderately permeable to water, but very poorly so to salts. This quality has been termed *semi-permeability*, under the assumption that the membrane is wholly permeable to water and impermeable to salts. As this is not true, the terms *differential* or *selective permeability* are preferable, just as in non-living osmotic membranes (page 59).

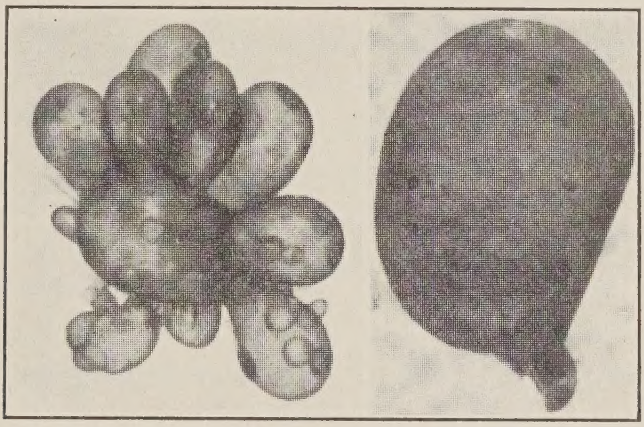

FIG. 35. *Valonia macrophysa,* left; *V. ventricosa,* right; natural size. (From F. C. Steward.)

The expression "power to select" is merely a convenient way to state what happens. If selective permeability is a matter, say, of relative rates of diffusion of ions, then obviously the cell in no way "selects." Actually, protoplasm does play a part in determining which ions shall enter. In either case, selection implies a purposeful act. Many terms in biology carry with them the concept of purpose; and, although it is well to avoid them, this is not always convenient or necessary.

The rapid entrance of alcohol, ether, and chloroform into cells as compared with the slow penetration of salts, and the even greater rapidity with which the light hydrocarbons, such as petroleum ether, enter, whereas the heavy hydrocarbons do not enter at all, are other illustrations of the selective permeability of protoplasm. These observations explain, for example, why gasoline is so very toxic to man, and a heavy petroleum oil is not; the gasoline is rapidly absorbed by the intestines. Certain dyes also show differential absorption; some stain the cell contents, others do not.

The first barrier to material entering a cell is the protoplasmic surface. The cellulose wall of plant cells is not taken into account, for it is generally so highly porous that no substantial resistance is offered to the passage of substances in solution.

THE PROTOPLASMIC MEMBRANE

It is usually assumed that the outer layer of a protoplast is an anatomically distinct membrane possessing certain qualities which differ from those of the inner protoplasm. Whatever properties are attributed to it, it should be realized that the protoplasmic membrane is capable of essentially the same changes and the same readjustments as is the bulk of protoplasm.

The cell membrane is similar in constitution to the inner protoplasm, from which it is usually quite indistinguishable; there would, therefore, seem to be some justification for the incorrect belief formerly held that no membranes surround cells. Moreover, it is difficult to visualize a membrane which is at times solid and again fluid. The surface layer of protoplasm is often in active motion, as it must be when a plasmodium or an amoeba is advancing. Inner protoplasmic membranes, those which surround vacuoles, are also often fluid and actively streaming. Those who support the membrane hypothesis, however, find no objection to a membrane becoming temporarily fluid, for it merely emphasizes that the membrane is of protoplasm.

If protoplasm is, even to a slight degree, amenable to the laws of pure liquids and true solutions, then it is evident that its surface is a *tension membrane*, such as exists at the surface of all liquids. A surface tension membrane, though hardly an anatomical structure, will yet have a retarding effect on the passage of certain substances.

The American physical chemist, Willard Gibbs, established the fact that substances having the quality of lowering surface energy will accumulate at the surface, and substances that raise the surface energy will not do so. This is the general rule and applies to all forms of surface energy, of which surface tension is one. Electrical energy at the surface of particles is another form, and particularly important for colloidal systems. Freundlich applied the Gibbs' law to one form of surface energy, namely, surface tension. The Gibbs-Freundlich rule states that substances which lower surface tension are more concentrated at the surface than in the interior, and substances which raise surface tension are less concentrated at the surface. A number of protoplasmic constituents, such as soaps and fats, lower surface tension. They must, for this reason, be more highly concentrated at the surface of cells than within. The protoplasmic membrane, therefore, will contain considerable fat material.

The progress of the advancing pseudopodium of an amoeba may be hastened by inserting a microneedle and rapidly moving it in the direction of flow. If this procedure is carried beyond a certain point however, the protoplasm suddenly gels at the periphery. Further movement of the needle is then accomplished only by tearing through the firm outer protoplasmic layer; the plasma sol has gelatinized and become a plasma gel. When the surface of protoplasm is in motion, it is fluid; when quiescent for any length of time, it is firm.

The firm condition of the surface layer of protoplasm may be demonstrated in another way, by microdissection. When a protoplast is isolated from its cellulose walls, it usually coagulates. In this state, the cytoplasm may be stripped off from the vacuole, leaving the latter free as a floating sac, thus indicating that the vacuole membrane, the so-called *tonoplast*, has become a coagulated layer which separates readily from the surrounding cytoplasm.

The presence of an inner, or vacuolar, membrane as well as an outer cellular one, brings up the question, which of the two is primarily responsible for permeability phenomena. Usually this question is answered by saying that both function, and presumably in a similar manner. There is, however, an ever increasing amount of evidence to show that they do not function in identical ways; this is clearly shown by the fact that the concentration of any one kind of ion is often not the same in the protoplasm and the vacuolar sap. S. C. Brooks has shown that rubidium is taken up by cells much faster than it appears in the sap.

Having acquired sufficient evidence to justify the assumption that the protoplasmic membrane has an identity of its own, it is possible to proceed to a consideration of those peculiar qualities which give to it the remarkable property of selective permeability.

THE LIPOID HYPOTHESIS

The first constructive suggestion on the cause of selective permeability of cells was the *lipoid hypothesis* of Overton. Carbon dioxide, alcohol, ether, acetone, chloroform, and ammonia enter the cell very rapidly, ammonia about fifty times as fast as potassium. Most of these are fat-solvents. Equilibrium between the concentrations of fat-solvents without and within the cell is reached in a few seconds. Salts, on the contrary, enter so very slowly that, if equilibrium is reached, it is only after a long time. Some quality of the plasma membrane thus permits fat-solvents to enter rapidly while retarding salts. Overton considered this quality to be due to the presence in the membrane of fat-like substances or *lipoids*. The term "lipoid," meaning "fat-like," is more freely used by biologists than by chemists. It includes such common cell constituents as lecithin and cholesterol, the former a phosphatide and the latter a sterol. Fats are particularly efficient in lowering surface tension; therefore, according to the Gibbs-Freundlich rule, they will be concentrated at the surface. As protoplasm is rich in fats, its outer layer will contain more of them than the inner protoplasm.

Overton limited his theory to those fat-solvents which are slightly soluble in water, as are ether and chloroform. This met the objection that, as "like dissolves like," an oil such as olive oil should penetrate a lipoid membrane; yet actually olive oil does not enter cells. A further criticism directed against the lipoid hypothesis was that essential salts and other necessary water-soluble substances cannot pass through a layer of fatty material. The answer to this is that, as the lipoids under consideration are slightly water-soluble, they offer channels of passage for substances in aqueous solution. The lipoid hypothesis, then, adequately explains certain permeability phenomena, but it is not sufficient as an all-inclusive theory.

THE PROTEIN HYPOTHESIS

A protoplasmic membrane which is primarily of protein will permit water-soluble substances to pass through. Evidence of a protein cell membrane is to be had from a number of observations; only one need be mentioned. The surface of protoplasm coagulates at death; proteins coagulate, but oils do not.

THE MOSAIC HYPOTHESIS

Nathansohn made the suggestion that in order to meet all conditions the cell membrane must be a *mosaic* of fat and protein. A membrane of heterogeneous composition would, of course, furnish channels of entrance for a variety of substances, each requiring specific solvents. That the protoplasmic membrane is both fat and protein seems probable, but the two are not necessarily intermixed as in a mosaic. Various observations and experiments indicate that the fat is distributed as a film on the outside of a more substantial protein layer.

THE SIEVE HYPOTHESIS

Interpretations of selective permeability may be based on either chemical or mechanical qualities. Those hypotheses so far advanced deal with the chemical behavior of the membrane; the following are mechanical interpretations.

Observations on the osmotic behavior of cells indicate that sugars enter less freely than salts. If the protoplasmic membrane is a sieve, the more ready entrance of salts may be interpreted on the basis of the relative sizes of the salt and sugar molecules, for salt molecules are small and sugar molecules large. A membrane with pores the diameters of which are intermediate between those of salt molecules and those of sugar molecules will permit salts but not sugars to pass through. There is considerable evidence to support the *sieve hypothesis* of protoplasmic permeability. All living membranes are rather freely permeable to water, less so to salts, only slightly so to sugars, and almost impermeable to proteins. The ease with which these compounds penetrate cells is thus directly proportional to their molecular diameters.

Certain artificial membranes show the same relationship between selective permeability and molecular size. The Traube precipitation membrane (page 60) permits water to pass freely but retards the entrance of salts. Water and salts penetrate collodion membranes readily, but neither proteins nor colloidal particles pass through in quantity.

It is very probable that the selective permeability of some membranes is purely a question of pore size; but, although the living protoplasmic membrane may possibly function in part as a sieve, there are experimental findings contrary to this assumption. In particular, there is no parallelism between the sizes of the molecules and the relative rates of entrance of certain alcohols, sugars, and other substances into the living cell. The following table (from Collander) indicates this:

SUBSTANCES	RELATIVE PERMEABILITY OF THE PLANT CELL	MOLECULAR VOLUME
Methyl alcohol	125.0	8.2
Ethyl alcohol	71.0	12.8
Glycerol	1.3	20.6
Urea	1.1	13.7
Glucose	1.02	37.5
Saccharose	1.0	70.4

With the exception of glycerol, the smaller the molecule the more rapid the entrance; yet the sizes of the molecules of the last three compounds have the approximate ratio 1 : 3 : 6, whereas their rates of entrance are practically the same.

S. C. Brooks suggests that the pores of the membrane are not of a fixed size but are capable of adjustment, which may explain the occasional anomalous behavior of the membrane.

The mechanism of a selectively permeable sieve may be interpreted in terms of the orientation of molecules. Protein and certain fat molecules are known to be elongated; and molecules of such a shape usually exhibit polarity, that is to say, their ends differ. This means that they will become oriented at an interface. Little material can pass between long molecules compactly arranged, but a loose orientation will result in a freely permeable sieve.

THE EMULSION HYPOTHESIS

The coarser structure of protoplasm is that of an emulsion. A number of investigators assume that the finer ultramicroscopic structure of protoplasm is also an emulsion.

An oil-in-water emulsion is freely permeable to water-soluble substances because water is the continuous phase; and a water-in-oil emulsion is permeable to oil-soluble substances because oil is the continuous phase (page 20). These facts, and a certain analogy between the permeability of protoplasm and the behavior of emulsions in the presence of sodium and of calcium, led Clowes to regard the protoplasmic membrane as a fine emulsion, at or near the reversal point.

There are several points which lend favor to the emulsion hypothesis of permeability, notably Clowes' discovery that the proportion of sodium and calcium which keeps an emulsion at the reversal point is the same as that which exists in physiologically balanced solutions, such as sea water and milk. Dixon finds support for the emulsion hypothesis of permeability in the similarity between the electrical conductance of the plasma membrane and that of emulsions when in the presence of electrolytes.

Some fundamental objections to the emulsion hypothesis of permeability exist, however. There is, for example, no evidence whatever that phase reversal occurs in protoplasm; nor will the sodium ion reverse any known emulsion. Calcium reverses emulsions from oil-in-water to water-in-oil, and sodium will retard this effect; but sodium will not reverse emulsions from water-in-oil to oil-in-water. This is accomplished only by the hydroxyl, or OH anion.

THE ELECTRICAL HYPOTHESIS

The electrical properties of matter have come to play a very important part in the interpretation of many vital phenomena. A selectively permeable membrane bathed in solutions of electrolytes is the seat of electrical forces. This being true, the penetration of electrically charged particles will be influenced, if not determined, by the charge on the membrane. Theory requires that a positively charged membrane repel positively charged particles, and experiment shows that positive ions do not pass through positively charged membranes whereas negative ions do. The charge on protein surfaces can be reversed by bathing in an acid medium, which gives a positive charge, or in an alkaline medium, which gives a negative charge. This is possible because the membrane is *amphoteric*, as are most proteins (page 183). When positively charged, as in an acid medium, the membrane permits anions to pass through; and when negatively charged, as in an alkaline medium, it permits cations to pass through.

OTHER HYPOTHESES

A number of other suggestions on the mechanism of the plasma membrane have been made. Thus, Czapek thought semi-permeability to be a matter of surface tension—any substance which will lower the surface tension of protoplasm below its normal average value will enter. Warburg thought semi-permeability to be a matter of adsorption—the more readily a substance is adsorbed by the surface of protoplasm, the more rapidly does it enter. Donnan sought to interpret selective permeability in terms of electric equilibrium. If a membrane separates two salts, all ions of which except one are freely diffusible through a membrane, then, at equilibrium, the products of the diffusible ions on the two sides of the membrane are equal. The theory gives the distribution of the diffusible ions at equilibrium but presents no interpretation of the selective permeability of the membrane.

At the present time, it is impossible to say which of the foregoing hypotheses offers the most likely explanation. Possibly all play a part; solubility, pore size, electric charge, surface tension, adsorption, and electric equilibrium.

METHODS OF INVESTIGATION

Ways of determining permeability changes in the cell are several. The most satisfactory visual proof of a change in permeability is to be had from dyes added to the surrounding solution. If they enter more or less readily after a cell has been treated with a salt or other substance, the permeability of the protoplasmic membrane has been altered.

The loss of pigments within the cell is also a very satisfactory way to note permeability changes. The red coloring matter in the beet is not lost when a slice of beet is put in cold water, but immediately diffuses out when the beet tissue is put in hot water. Heat has increased cell permeability causing loss of the dye. The loss of the red pigment hemoglobin is the most common method of observing permeability changes in the red blood cell: the process is known as hemolysis, and may occur naturally.

The ease with which cells are killed by an agent diffusing into them after treatment is still another method of investigation of change in permeability. A 10 per cent solution of alcohol will kill the cells of *Elodea* leaves in 30 minutes. After treatment with a reagent the cells will be killed by the same concentration of alcohol in a greater or less time, depending on whether the reagent has caused an increase or decrease in permeability.

Determinations of the electrical properties of membranes represent a very refined technique in permeability studies. It has long been known that the protoplasmic membrane resists the passage of an electric current. This electrical resistance is rather high and changes with change in environment or internal state of the cell. Injury or death may bring about as much as a 90 per cent decrease in resistance. That the protoplasmic surface offers resistance to the passage of an electric current has been assumed to be proof of the presence of a membrane. Changes in surface resistance in the seaweed, *Laminaria,* were used by Osterhout as indications of changes in permeability. With this earlier work as a starting point, L. R. Blinks determined the resistance of the protoplasmic surfaces of the green algae, *Nitella, Valonia,* and *Halicystis.*

Electrodes may be applied to two points on the surface of the exterior of the cell, as in *Nitella*, or the current may be measured by putting fine capillary electrodes into the cell, as in *Valonia* and *Halicystis.* Using these methods, Blinks found the resistance of the surfaces to have the high values of 100,000 to 700,000 ohms per sq cm of protoplasmic surface, with an average of 250,000 ohms per sq cm in the case of the fresh-water plant, *Nitella,* and 10,000 ohms per sq cm for the salt-water plant, *Valonia.* The lower resistance of the marine plant is probably due to higher salt content of the protoplasm. The protoplasmic capacity in both *Valonia* and *Nitella* is roughly of the order of 1 microfarad per sq cm of cell surface. As yet the ionic species responsible for these and other electrical effects is unknown, although potassium is known to inhibit recovery from excitation.

PROBLEMS IN PERMEABILITY

There are permeability phenomena which are capable of interpretation in terms of the foregoing theories, whereas others defy all attempts to understand them. There are differences in the rate of penetration of very similar ions; thus, in the following series, rubidium enters the cell most rapidly and calcium least rapidly.

$$\mathrm{Rb} > \mathrm{K} > \mathrm{Na} > \mathrm{Li} > \mathrm{Ng} > \mathrm{Ba} > \mathrm{Sr} > \mathrm{Ca}$$

All the monovalent ions are on the left of the center and all the bivalent ones are on the right; this would suggest that valence, or combining power and properties associated with it, such as electric charge, may be responsible. Much has been made of this assumption; yet there are differences among the members of each valence group, i.e., the monovalent cations present a series among themselves, and the bivalent ones do the same.

Certain membranes are more permeable to the cation of a salt than to the anion; thus, the apple skin is more permeable to potassium than to chlorine. The electric charge of the membrane may be responsible here.

The seed coat of the chestnut, like the digestive tract of animals, is more permeable to water in one direction—from without to within—than in the reverse direction. Such one-way permeability is a particularly baffling problem, but it may find an interpretation in the phenomenon known as electroendosmosis, the one-directional streaming of water through capillaries under the influence of an electric potential (page 20). The pores of the membrane are the capillaries, but there is doubt as to the existence of sufficient potential to accomplish the streaming.

The alga, *Valonia* (Fig. 35), already referred to, presents a three-fold problem. There is first the question of how a concentration of potassium forty times that in the surrounding sea water has been stored in the cell, the potassium having apparently diffused against a concentration gradient. Next, there is the question of why potassium is stored and sodium not; and finally, there is the extraordinarily different behavior of *Halicystis*, a close relative of *Valonia.*

The first part of the general problem is more easily interpreted if, as S. C. Brooks points out, the storing of potassium is viewed not as an accumulation but as an exchange of ions; from this point of view, a balance is maintained on the two sides of the protoplasmic membrane. The accumulation is then only one of potassium in proportion to sodium and not an excess of ions.

Storing salts and other substances against a concentration gradient is not uncommon in organisms, and presents one of the most interesting and baffling problems in physiology; for example, certain dyes when allowed to perfuse into the liver of a frog in very dilute concentrations are given off four hundred times more concentrated than the original solution.

The second part of the general problem of selective permeability in *Valonia* is the storing of potassium far in excess of the surrounding concentration, whereas the concentration of sodium is but one-fifth that in the sea. For this there is as yet no satisfactory explanation.

The third part of the general problem has to do with the different behavior of *Halicystis.* Though a close relative of, and growing in the same water with, *Valonia,* it stores more sodium than potassium. Any theory explaining the behavior of the one alga would not fit the other.

Satisfactory explanations of many permeability phenomena are lacking; certain others, however, though seemingly equally perplexing, are capable of interpretation. The four hexose sugars, glucose, galactose, fructose, and mannose, pass through natural membranes of living cells at different rates, yet all have molecules of the same size. What appears to be a case of anomalous behavior is not that at all because the problem is not one in permeability. All four sugars are able to enter at the same rate; but they are consumed by the cell at different rates. Glucose is consumed in respiration, but mannose is not. Those sugars rapidly used will enter more rapidly in order to maintain equilibrium. The slowly entering sugars penetrate the membrane slowly, not because of selective permeability, but because they are not used in quantity by the cell; in other words, the rate of entrance is determined by selective metabolism and not by selective permeability. This interpretation is ingenious and has some experimental evidence to support it, but it is rather an explanation than a general and established fact.

It has been suggested that only molecules enter cells. This speculation cannot be regarded as a general rule, but there are some experimental facts to support it, involving certain weak electrolytes only, e.g., for carbon dioxide and ammonia in solution. That only molecules enter cells cannot represent a general condition, for the plant deals with ions both in the soil and in its body. New theories indicate that salts are fully dissociated even in high concentration (page 97). This being true, then salts in the relatively dilute concentrations in which they occur in the soil must be fully dissociated; consequently, the essential elements are to be expected to enter as ions.

The mechanism of permeability control and the effects of environmental influences upon it are among the most fundamental of plant processes. The entrance of salts, the translocation of sugars, and the storage of foods are problems in permeability. Plant diseases involve changes in cell permeability. Studies on protoplasmic behavior indicate that one of the first signs of collapse in the organization of a cell is a breakdown in permeability control.

Comparison of the permeability control of tropical and temperate plants illustrates the practical importance of permeability studies and the perfection of adjustment between a plant and its environment. The temperature required to destroy the semipermeability of cells in most tropical plants, as determined by the setting free of the cell sap, is –16°C; and in plants growing in Canada, it is –70°C.

There is also the important matter of water control. The cell membrane and the protoplasmic jelly are not freely permeable to water. Höfler has emphasized that the movement of water into and out of cells is as delicately controlled as that of salts and more complex substances.

Scarth has shown that there is a close relationship between resistance to freezing, or winter hardiness, and permeability. Resistance to cold is accompanied by an increase in cell permeability which protects cells against the mechanical injury due to frost. The higher the permeability to water, the less danger there is of intracellular freezing and damage due to the formation of ice crystals in the cells.

As a final word, it will bear repeating that cells exhibit individual permeability properties, and that in all cells there is permeability control and change. Selective permeability is not fixed. There is change and adjustment, for protoplasm is alive, dynamic, and not static. Nor is the permeability of one cell necessarily like that of another; certainly not when they come from different tissues, or from different organisms. Experimental results and hypotheses based on one type of material do not necessarily hold for other types. Certain few and rather general rules appear to hold; thus, sodium increases permeability because of its dispersing effect on protoplasm, whereas calcium decreases permeability because of its coagulating effect. There is, however, no universal law of permeability.

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CHAPTER XII

PHOTOSYNTHESIS

The greatest of all chemical processes is *photosynthesis.* It holds this position because it is the first step in the natural production of foods upon which all life depends. There are a few organisms which use inorganic matter as a source of energy, as do the iron and sulphur bacteria. The vast majority of plants and animals must rely upon the process and products of photosynthesis in order to sustain life.

The storage of solar energy by the plant is of wider benefit to mankind than the mere supplying of food by photosynthesis. Fuel, whether food, wood, coal, or oil, is but stored sunlight, collected by the plant and fixed in the form of elementary or incompletely oxidized carbon. Sugars and fats serve us for internal heat, and wood, coal, and oil for external heat.

Photosynthesis comprises that series of reactions in which organic substances are formed from inorganic ones, with sunlight as the source of energy and the green pigment in plants as catalyst. Formerly, only living organisms were thought capable of synthesizing food from inorganic material. Plants were presumed to possess a special life force, a *spiritus vitae*, which gave them the capacity to synthesize organic matter, an accomplishment that man could not hope to duplicate in the laboratory. This assumption was the basis of *organic* chemistry, a chemistry considered to deal with substances produced only by organisms. The assumption collapsed when Wöhler synthesized urea, in 1828, while working as a student in the laboratory of Berzelius. The artificial synthesis of urea marked the beginning of substantial opposition to the philosophy of *vitalism.*

The ancients looked upon the soil as the source of a plant's nutrition, as any tiller of the soil might do. They could not know, any more than did Van Helmont sixteen centuries later (page 7), that one of the most important constituents entering into the photosynthetic process comes from the air; that constituent is the element carbon, supplied as carbon dioxide.

The other of the two primary materials for the making of food in plants is water, and this comes from the soil.

One of the first constructive suggestions concerning photosynthesis was made by Malpighi who said as early as 1675 that leaves were the seat of food production in plants. Then followed the contribution of Stephen Hales, who in 1727 conceived that some of the material used by plants comes from the air and is absorbed by the leaves. He also realized that light is a factor in plant metabolism. The experiments of Priestley and Ingen-Housz showed that plants thrive in "foul" air and purify it. Shortly afterward, Lavoisier established the presence of oxygen in the air, discovered the composition of carbon dioxide, and deduced therefrom the nature of combustion. Ingen-Housz was then able to carry interpretations of photosynthesis and plant respiration still further. In 1779 he demonstrated that only green plants purify the air. De Saussure proved that the volume of oxygen given off is equal to that of the carbon dioxide taken in, showing the ratio between the two to be unity. Boussigault reestablished all these results in a series of accurately performed experiments and rounded out the problem by demonstrating that photosynthesis starts as soon as the plant is illuminated and ends when the plant is placed in darkness.

These pioneer investigations yielded the fundamental facts of photosynthesis. Only details have been added. The working out of the details, however, presents a far greater task than that which confronted Priestley and Ingen-Housz.

Carbon dioxide and water are combined in the plant in such a way as ultimately to produce sugar. This synthesis occurs only in the green parts of plants and in sunlight, which is the energy governing the reaction. These facts are not questioned; but of the intervening steps which lead from carbon dioxide and water to the final product, sugar, very little is known.

Photosynthesis is a term of American origin widely used in France, but in England and Germany it has been replaced by the expression, *carbon assimilation,* or just *assimilation.* Which term is used depends entirely on whether emphasis is placed on synthesis under the stimulus of light energy or on the binding of carbon.

Ways of demonstrating photosynthetic activity are simple if exact quantitative results are not required. An aquatic plant placed in sunshine will emit bubbles of oxygen, which may be collected in a test tube inverted over the outlet of a funnel (Fig. 36). The amount of gas produced in a given time can be deter-

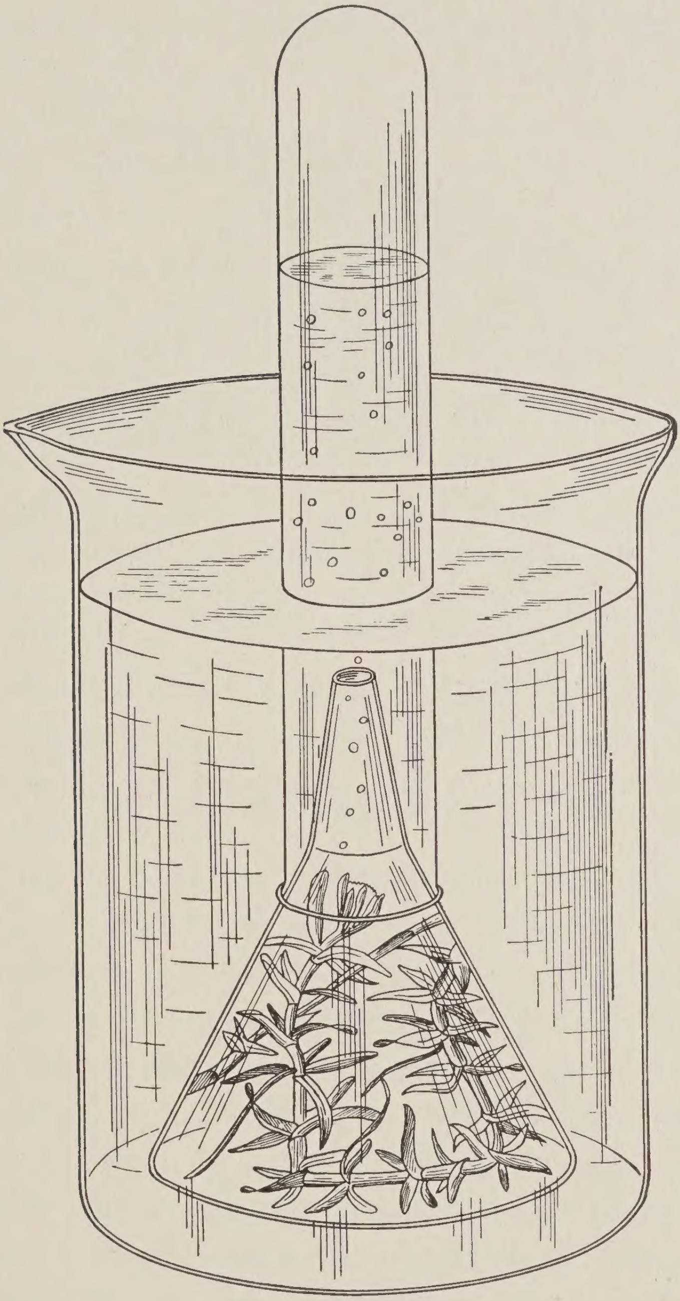


FIG. 36. A simple means of collecting the oxygen formed as a result of photosynthesis.

mined from the volume of water displaced. That the gas is oxygen can be proved by the fact that a glowing match stick will burst into flame when thrust into it.

A leaf covered in part with tinfoil and placed in the sun for a few hours will, when stained with iodine, show that no starch was formed where no sunlight penetrated (Fig. 37).

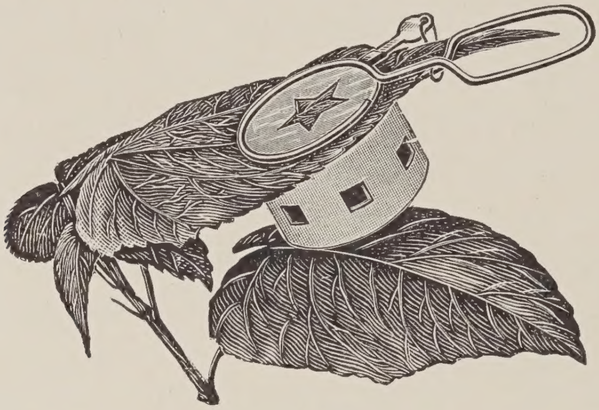


FIG. 37. A light screen for demonstrating the formation of starch. (Courtesy of the Chicago Apparatus Co.)

The factors determining and influencing photosynthesis may now be discussed; first, the four major ones, sunlight, chlorophyll, water, and carbon dioxide.

THE MAJOR PREREQUISITES

Sunlight. *The solar spectrum* includes all of visible light and extends into the ultraviolet and the infrared, being part of the larger *electromagnetic* spectrum. The longer heat rays lie beyond the infrared, and the shorter X-rays beyond the ultraviolet. The visible region of the light spectrum extends from red to violet, which in terms of wave length is from about 7500 A. U. (0.75 μ) to 4000 A. U. (0.40 μ). Plants may use small amounts of the energy resident in the invisible portions of the solar spectrum, but the visible light is its chief source of energy. That most plants do not make use of, or at least do not require, ultraviolet light is evident from the fact that plants grow well in greenhouses where little ultraviolet penetrates the glass. Ultraviolet radiation has a pronounced effect on photochemical systems, for example, on the photographic plate. This would lead one to expect it to function in photosynthesis. However, a form of energy which activates one type of chemical system need not do the same for another.

There is no conclusive evidence that ultraviolet light affects photosynthesis, although reports are occasionally made that it has an effect on other forms of plant activities; it is said to retard growth and bring about anthocyanin, or pigment, formation. Important in this connection is the very low penetrating power of ultraviolet light. Although it affects the leaf surface—the epidermis, hairs, and scales respond energetically to ultraviolet—its effectiveness is gone by the time it has penetrated the epidermis. The palisade and mesophyll tissues, where the chloroplasts lie, accordingly receive none of it.

The infrared radiation appears to be about as ineffective as ultraviolet as an activator of photosynthesis. Again the statement can be taken only as a general rule, for some algae use infrared rays, and conditions determine their usefulness. The temperature coefficient is a factor; some plants can use the heat energy of the infrared, as is true of certain other photochemical reactions.

The most effective wave lengths or colors of the visible spectrum which activate photosynthesis can be ascertained either by subjecting a plant to the various colors of the spectrum (Fig. 38) or by observing which colors are absorbed by the leaf.

Green light is not absorbed in quantity, but, being reflected, causes the color of the leaf to appear green. If white light is permitted to penetrate a solution of chlorophyll, or a layer of several leaves placed at the end of a tube, the last trace of color coming through is deep red; therefore the rest of the spectrum, except green, has been absorbed. A colloidal solution of gum-mastic illustrates the situation: the Tyndall cone formed (see page 17) in such a solution is green at the end where light enters, the violet having been immediately absorbed by the glass container or the solution; the next color seen is yellow, then orange, and finally red; only the deepest red comes out at the far end. In a similar manner chlorophyll acts as a color screen. If a light spectrum is viewed through layers of an alcoholic solution of chlorophyll of increasing thickness, a band in the violet and red are soon formed, indicating absorption. Later bands appear in the yellow and orange. All broaden until only deep red still penetrates (Figs. 39, 40). The spectra of the various constituents of chlorophyll differ.

A series of experiments in which plants are subjected to light of different wave lengths and the resulting photosynthetic activity ascertained in terms of the volume of oxygen produced, amount of starch formed, or the increase in weight established will yield the curve in Fig. 41. When activity is compared with the absorption spectrum of a leaf, it will be seen that the rate of assimilation is greatest in the red (690 mμ), depending somewhat on the composition of the light and the kind of plant.

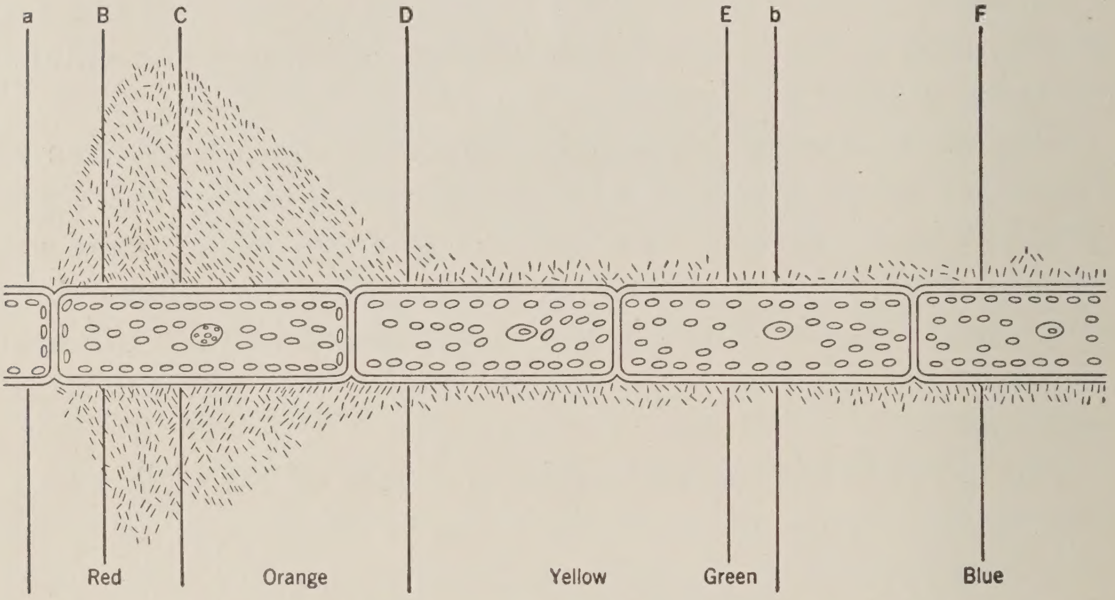


FIG. 38. Collection of bacteria along the filament of a green alga subjected to the rays of the spectrum: the greatest number of bacteria are in the red region of the spectrum because the greatest amount of oxygen is being formed there, thus demonstrating that photosynthetic activity is greatest in the red band of the spectrum. (From W. Pfeffer.)

Misunderstanding often arises in regard to the wave lengths of light used by plants. This is likely to be due to failure to distinguish between the two distinct categories into which the effects of light on plants fall. Light may affect photosynthesis on the one hand, or such other plant activities as growth, reproduction, and cell permeability on the other. It is necessary to distinguish between these two types of effects of light and not allow the result of one to be ascribed to the other. Funke has shown that the development of plants in blue light is very similar to development in full daylight, whereas red and green have the same effect as darkness. In blue light the plants approach the normal state; in red they become blanched or etiolated. These results seem to contradict those showing that assimilation is greatest in red light and less in blue; but there is no real contradiction. In red light, plants remain underdeveloped; in blue light, they grow to maturity; in other words, photosynthetic effect is due to red rays, whereas the blue rays have a growth-stimulating effect. It is possible that the blue rays cause the formation of growth-producing hormones. Moreover, cell permeability is not the same in different parts of the spectrum. It may be affirmed, therefore, that plants use in one way or another

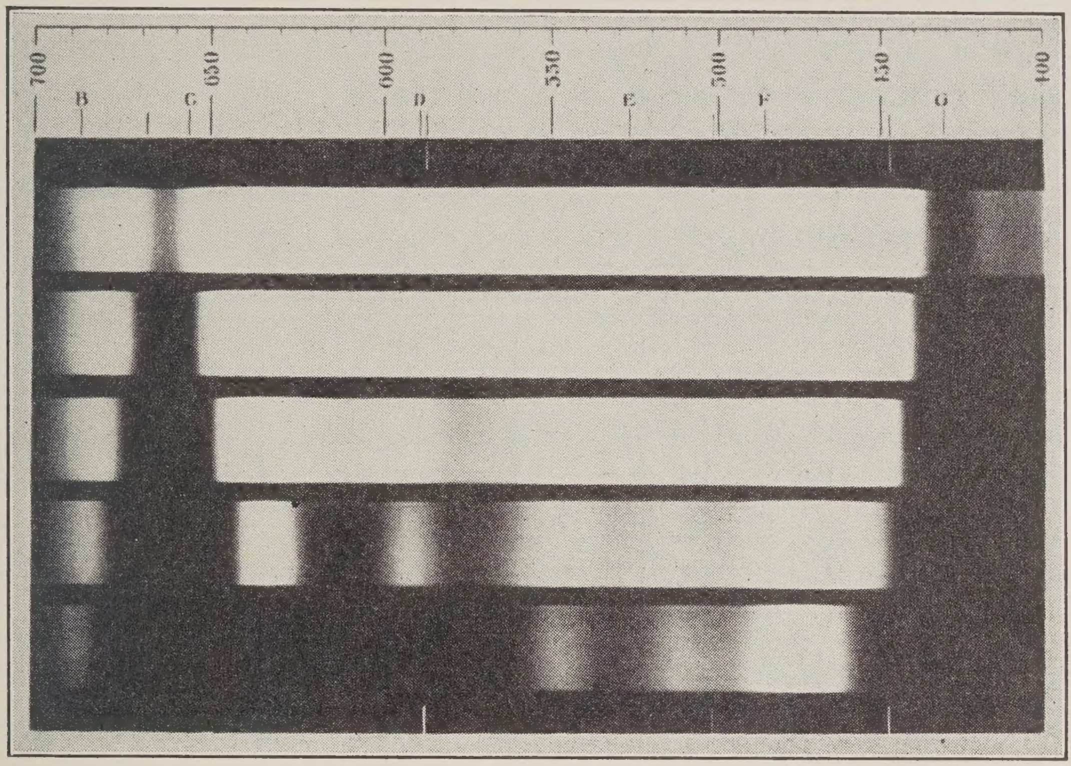


FIG. 39. The spectrum of chlorophyll *b* in alcohol. (From Willstätter and Stoll.)

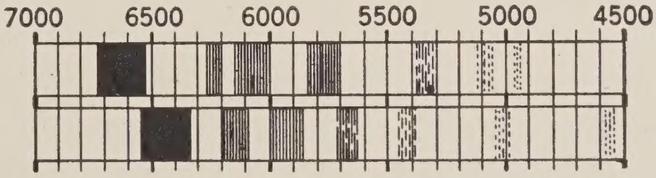


FIG. 40. Spectra of chlorophyll *a* and *b* in ether. (From P. Rothemund.)

practically all parts of the spectrum, and only develop normally when they receive all parts.

Chlorophyll. The catalyst responsible for the photosynthesis of food is *chlorophyll.* That chlorophyll brings about the synthesis of sugar from carbon dioxide and water cannot be proved with absolute certainty; but, as photosynthesis takes place only in cells containing chlorophyll, there is every likelihood that the activator responsible for photosynthesis resides in this substance.

The constituent of chlorophyll which is the activator of the synthetic reaction has not yet been isolated. There are at least two components of chlorophyll. The enzyme *chlorophyllase* has been found in leaves, but no evidence so far produced indicates that it plays a catalytic part in photosynthetic reactions. The splitting of phytol from chlorophyll through hydrolysis—if water is the solvent—or alcoholysis if an alcohol is the solvent —is the only action ascribed to it.

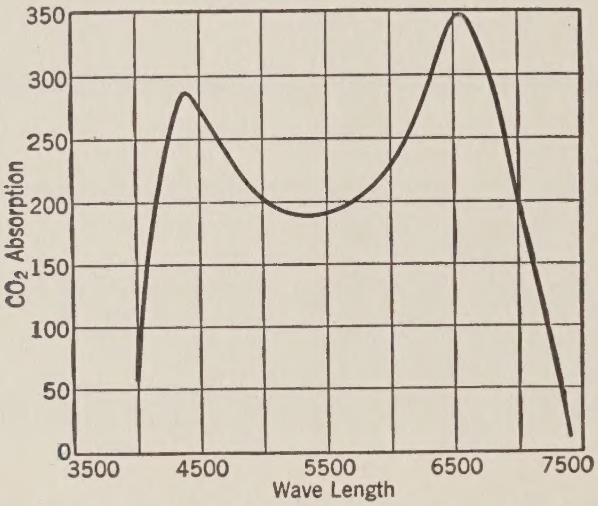


FIG. 41. Curve illustrating rate of carbon-dioxide assimilation in wheat plants at different wave lengths of light, from which it is seen that the red rays are the most effective, blue rays second, green and yellow rays useful, whereas infrared and ultraviolet contribute nothing to assimilation. (After W. Hoover, from E. Johnston.)

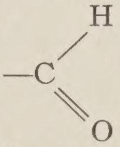
Chlorophyll may be extracted from leaves by crushing or boiling them in acetone or alcohol. Extractions in alcohol may cause secondary reactions, e.g., chlorophyll → methylchlorophyllide; therefore, acetone or ether solutions are preferred for spectrographic (Fig. 40) and other studies. In transmitted light, the solution is deep green in color and intensely fluorescent. If benzine is added to the acetone or alcohol solution, two layers are formed, an upper one of benzine, green in color, and a lower one of acetone or alcohol, yellow in color. From this it is evident that the green coloring matter from the leaf—as represented by the crude extract—contains a yellow pigment as well as a green one. More careful analysis reveals that there are two green and two yellow pigments; these have been given the names of chlorophyll *a*, chlorophyll *b*, *xanthophyll,* and *carotin.* The formulae of these pigments are:

Chlorophyll *a*.	$C_{55}H_{72}O_5N_4Mg$
Chlorophyll *b*.	$C_{55}H_{70}O_6N_4Mg$
Xanthophyll.	$C_{40}H_{56}O_2$
Carotin.	$C_{40}H_{56}$

The exact role played by each of these pigments is not yet known, although it seems likely that the catalytic powers lie only in the green ones, the chlorophylls proper.

The natural solvent of chlorophyll is as yet undetermined but it is probably of a resinous or waxy character.

The two yellow pigments, xanthophyll and carotin, are always found with chlorophyll, but there is no evidence that they play any part in photosynthesis. One of them, however, has attained a very important position among nutritional substances: carotin is the precursor of vitamin A.

It is the green pigments, or a constituent of them, which are responsible for the synthesis of sugar from carbon dioxide and water. The two are very similar. Chlorophyll *a* has two hydrogen atoms more and one oxygen atom less than chlorophyll *b*. Chlorophyll *b* carries a formyl group,  $-\mathrm{C}\begin{smallmatrix} \diagup \mathrm{H} \\ \diagdown \mathrm{O} \end{smallmatrix}$ in place of one of the methyl groups of chlorophyll *a*. Each has one magnesium atom linked through two nitrogens to a complicated ring system of carbon, hydrogen, oxygen, and other nitrogen atoms.

It is possible that chlorophyll is not alone responsible for photosynthesis. The fact that photosynthetic activity has been found not to be proportional to the amount of chlorophyll present suggests this. Other catalysts, in particular non-pigmented ones, may, therefore, be in part responsible for the primary synthesis of food in plants.

The synthesis of chlorophyll is probably accomplished by protoplasm, or a specific catalyst present in it, and sunlight is the source of energy for this synthesis just as it is in photosynthesis where the chlorophyll becomes the catalyst. That chlorophyll is formed only in the presence of light is shown by the etiolation of leaves when plants are left in darkness; that is, the leaves become yellow. If their deterioration is not too great, however, they reassume their green color on being returned to sunlight. Light of medium intensity is best. Full sunlight is as ineffective as very weak light. Temperature is also a factor. The elements essential as ingredients in the formation of chlorophyll are carbon, hydrogen, oxygen, nitrogen, and magnesium. Magnesium is the only element among the five which is not commonly present in higher organic compounds, such as the proteins.

Another important element involved in the synthesis of chlorophyll, but not included in its chemical constitution, is iron. It, apparently, is the catalyst in protoplasm responsible for the production of chlorophyll. With deficiency in iron, leaves turn yellow, that is, become *chlorotic* (pages 99-106) in distinction from an *etiolated* or blanched leaf which has become yellow because of the absence of light.

The chemistry of the synthesis of chlorophyll is not fully known, but some work has been done which has led to an insight into the matter and added further evidence in support of the hypothesis that there is a phylogenetic relationship between chlorophyll and hemoglobin. Iron is necessary for both chlorophyll and hemoglobin—for the synthesis of the former and as a constituent of the latter. Chlorophyll and hemoglobin also contain a common atomic group, the pyrrol ring (C_4H_4NH). Hemoglobin consists of a protein, globin, and of a pigment component, hemochromogen. The latter can be isolated from hemoglobin in form of its complex iron chloride, hemin. Hemin has in its molecule a similar arrangement of atoms as chlorophyll, except that iron takes the place of magnesium. By eliminating the iron in hemin, the same compounds (porphyrins) result as when magnesium is eliminated from chlorophyll. Chlorophyll, hemoglobin, the bile pigment bilirubin, and all important body pigments of both plants and animals may have a common origin, although no real significance can be attached to the kinship other than an evolutionary one. It has been found possible to remove the magnesium atom from chlorophyll and to split off methyl alcohol and phytol from components *a* or *b*. By partially decomposing chlorophyll, various magnesium-free compounds called *porphyrins* are produced. Decomposition by other methods results in magnesium-containing compounds called *phyllins*.

All porphyrins consist of a ring system composed of twenty carbon atoms, fourteen hydrogen atoms, and four nitrogen atoms. This fundamental ring is called *porphin*; it can be synthesized from pyrrole and formaldehyde. Porphin and magnesium yield *porphin-phyllin*, which is the simplest chlorophyll-like compound known. Its synthesis has been accomplished by Rothemund. Thus has the halfway mark been reached in the artificial production of chlorophyll. The next step in man's progress toward the artificial creation of life's greatest synthesizer will be the production of *protochlorophyll*, the natural precursor of chlorophyll. Protochlorophyll is a colorless compound which can be extracted from plants grown in the dark.

A number of modified forms of chlorophyll occur in nature which function catalytically but act upon different substances; thus, certain bacteria contain an acetylated chlorophyll, the bacterio-chlorophyll. The sulphur bacteria synthesize organic compounds from carbon dioxide and hydrogen sulphide. Sulphur or sulphuric acid are by-products instead of oxygen. The sulphur bacteria carry on this synthesis by using infra-red light (7500-9200 A), with an optimum growth temperature of 35°C, whereas higher plants use visible light (4000-7500 A) and have an optimum temperature for growth of 26°C.

Experiments on the reaction of animals to light-sensitive pigments have shown that fluorescing dyes produce harmful effects when injected into animals. The porphyrins are such dyes; when injected into rats, the animals show no ill effects while in the dark, but die on exposure to light. Photosensitization takes place in light. Similar effects were obtained with man; illness resulted when the patient remained in light. Paramecia treated with acridine at a concentration of 1-20,000 were killed in six minutes in sunlight, in one hour in weak light, and were unharmed in darkness.

Such experiments have their practical value; thus black pigs can eat buckwheat with impunity, but white pigs which have eaten it die in a short time unless kept in the dark. Investigations along these lines will lead to a better understanding of the beneficial and harmful consequences of sunlight on man.

Carbon Dioxide. Another primary prerequisite for the synthesis of organic matter in plants is carbon dioxide.

Gas first gains entrance to a plant through the pores or *stomata* of the leaf (page 88). Substances seem to enter living cells only in solution; carbon dioxide, therefore, presumably enters the photosynthetic cells of leaves in the form of carbonic acid (H_2CO_3). There may, however, be some CO_2 gas in undissolved form, as in carbonated water, which forces one not to lose sight of the possibility of its entering as such. The amount of carbon dioxide in the air and its rate of penetration through cell membranes are so very low that other sources of supply, such as the soil, have been suggested; but apparently the plant makes no use of them, for in air free of carbon dioxide no assimilation takes place.

Water. Water is the fourth of the primary needs of the plant in carbon assimilation. It comes from the soil and reaches the leaves through the conducting vessels of the stem. The role of water in plant life is great and varied (page 84); outstanding is its role as one of the two elementary substances used in the manufacture of food by plants. How it plays this part is the subject of the following section. With the four primary conditions of photosynthesis now at hand, a discussion of the chemistry of the process may be entered upon.

THE CHEMISTRY OF FOOD SYNTHESIS

The chemistry of photosynthesis has long been a rich field for speculation. As photosynthesis is carbon assimilation, and as carbon dioxide is one of the elementary substances involved, the initial reaction may be the breakdown of carbon dioxide into carbon and oxygen ($CO_2 = C + O_2$). The carbon thus liberated would be available for combination with other substances to form organic compounds. More probably instead of the carbon being thus set free, it is immediately joined to hydrogen and oxygen. The simplest conceivable reaction of this sort is the direct combination of carbon dioxide and water:

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$$

That the plant, even with capacities far exceeding those of the chemist, accomplishes the synthesis of sugar in so simple a manner is, as W. O. James has pointed out, quite unthinkable. Reactions of the sixth order—those in which six molecules must collide simultaneously—are unknown, and their probability so slight as to preclude their occurrence with measurable velocity, as in photosynthesis.

Positive tests, obtained by applying iodine to leaves, suggest that starch is the first substance formed in photosynthesis; if true, then the following reaction would be a likely one:

$$6CO_2 + 5H_2O = C_6H_{10}O_5 + 6O_2$$

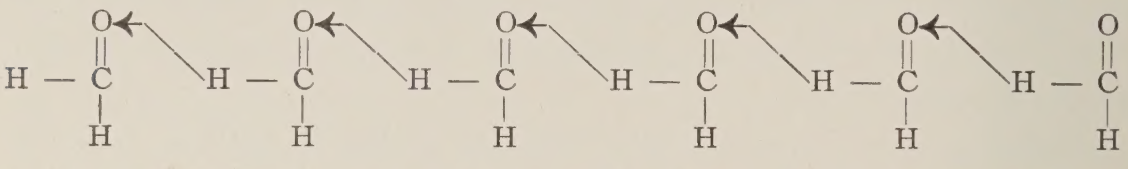
It has also been suggested that cane sugar is the first carbohydrate formed, and that hexoses are derived from it by hydrolysis; that is to say, first a disaccharide and then a breaking-down of it to the simpler monosaccharides. However, this is not the way syntheses usually proceed. Even still higher foods, oils, amino acids, and proteins, have been given support as direct products of photosynthesis. It is more likely that these organic substances are synthesized later elsewhere in the plant. That the simple sugars are first synthesized is supported by the manner in which foods are translocated. They are moved in a soluble form, for water is the medium, and the sugars are the most soluble of foods.

The discovery that a sugar-like substance is formed when dioxymethylene, which is a condensation product of formaldehyde, is heated in an alkaline solution led von Baeyer, in 1870, to advance the hypothesis that formaldehyde is the first product of photosynthesis and that sugar arises from it by condensation or polymerization. The equation would be:

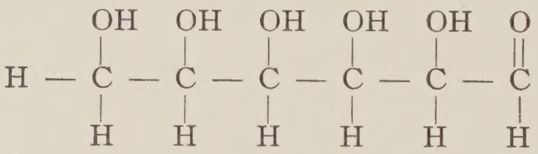
$$6CO_2 + 6H_2O = 6H_2CO + 6O_2 = C_6H_{12}O_6 + 6O_2$$

Whether or not a hexose sugar is the first product formed in the condensation of six formaldehyde molecules cannot be said. A somewhat more circuitous route is to be expected. Baeyer suggested dioxymethylene, but this substance does not exist. One can, of course, write the formula for it, as it is simply formaldehyde and water. While Baeyer postulated hydrated formaldehyde as an intermediary product, it is more likely that α-trioxymethylene is formed. This is the condensation product of three formaldehyde molecules.

Ignoring the possibility of secondary reactions, Baeyer's hypothesis may be represented in the following manner:



being the condensation of six molecules of formaldehyde, and yielding:



$$\begin{array}{ccccccccccccc} & & OH & & OH & & OH & & OH & & OH & & O \\ & & | & & | & & | & & | & & | & & \| \\ H & - & C & - & C & - & C & - & C & - & C & - & C \\ & & | & & | & & | & & | & & | & & | \\ & & H & & H & & H & & H & & H & & H \end{array}$$

Tests indicating the presence of aldehydes in plant leaves give support to the hypothesis of von Baeyer.

Further evidences contributory to Baeyer's suggestion are the following reactions which may take place when an electric discharge, as a source of energy instead of sunlight, is passed in the presence of carbon dioxide, oxygen, and water, individually or combined:

$$\begin{array}{rcl} 2CO_2 = 2CO + O_2 & \qquad & CO + H_2O = HCOOH \\ CO + H_2O = CO + H_2 & \qquad & 3O_2 = 2O_3 \\ H_2 + CO = HCOOH & \qquad & 2H_2 + 2O_3 = 2H_2O_2 + O_2 \end{array}$$

There are, however, other facts which stand against the Baeyer hypothesis; no formaldehyde can be detected in leaves, the aldehydes actually found being higher ones. Furthermore, formaldehyde is highly toxic to protoplasm; and as von Baeyer also postulated the reduction of carbon dioxide to carbon monoxide and oxygen in photosynthesis, another toxic agent, carbon monoxide, was added. Objection to the presence of both these substances can be met by assuming that condensation to sugar takes place immediately on the production of formaldehyde or carbon monoxide; or the formaldehyde may exist in some non-toxic, non-activated form, or may be bound to a protein or other constituent of chlorophyll. Erlenmeyer in 1877 suggested that carbon dioxide is not reduced to the monoxide but unites directly with water, forming carbonic acid which is reduced to formic acid and the latter to formaldehyde, in this manner:

$$CO_2 + H_2O = H_2CO_3$$
$$H_2CO_3 \rightarrow HCOOH + O$$
$$HCOOH \rightarrow H_2CO + O$$

Evidence in support of the formaldehyde theory has recently been added through the report of several workers, notably Benjamin Moore and E. C. C. Baly, that they have succeeded in producing formaldehyde through the action of light on water and carbon dioxide in the presence of certain suspended metallic oxides or colored metal salts which function as catalysts (page 150).

Feeding plants formaldehyde and noting if sugar is formed in the dark, on the assumption that a polymerizing enzyme is present, have led to a claim of positive results, and thus added more support to the von Baeyer hypothesis.

Assuming formaldehyde to be the first product of photosynthesis, the second major reaction would quite likely be the condensation of formaldehyde into a hexose sugar, $6CH_2O \rightarrow C_6H_{12}O_6$. There are thus available all the necessary basic radicles for building the simple carbohydrates:

$$6CH_2O \rightarrow C_6H_{12}O_6$$
$$2C_6H_{12}O_6 - H_2O \rightarrow C_{12}H_{22}O_{11}$$
$$x(C_6H_{12}O_6 - H_2O) \rightarrow (C_6H_{10}O_5)x$$

None of the foregoing reactions takes place spontaneously, so the question arises, for how many of them does chlorophyll serve as the catalyst? Failure to locate monosaccharides or sucrose in actively photosynthesizing leaves, whereas striking tests for starch are obtained, has led to the conclusion that starch is the compound first formed; yet this is unlikely, for if starch why not fats, proteins, and protoplasm, all of which occur in leaves? Furthermore, food is translocated in the form of sugar, and it would be poor economy on the part of the plant first to synthesize a higher product and then immediately break it down for transfer. Starch, fats, proteins, and protoplasm are built up *in situ*, that is to say, where they are used or stored, and therefore in every cell, most of which are without chlorophyll and sunlight.

No suggestion has been made on the possibility of chlorophyll entering into combination with the intermediate substances formed in carbon assimilation. A catalyst is ordinarily supposed not to unite with the substances it activates. The chemist, however, recognizes certain catalytic reactions in which molecular proportions of the catalyst are necessary. This can only mean that the catalyst enters into the reaction and in a strictly stoichiometric way. Willstäter and Stoll state that chlorophyll not only acts as a sensitizer but enters into the course of the chemical reactions. They considered that there is a union of carbonic acid, in which form carbon dioxide enters the cell, with the magnesium of the green pigment, forming a chlorophyll-formaldehyde-peroxide complex.

Another question still to be considered is the possible use of atmospheric nitrogen by the plant. Botanists have usually rejected this possibility except in special cases, as in the fixation of nitrogen by bacteria (page 167). If the leaf in its photosynthetic activities can utilize atmospheric nitrogen, the classical outline of photosynthesis will have to be modified. Nothing final can be said in regard to the vital synthesis of nitrogen-containing compounds; but, as artificial syntheses involving atmospheric nitrogen occur, it may be that atmospheric nitrogen is made use of by the plant. Baudisch has gone so far as to suggest a possible chain of reactions for such a synthesis; it involves the production of nitrosyl, $2(NO)H$, and hydrogen peroxide, H_2O_2, from atmospheric nitrogen, oxygen, and water, formed simultaneously with hexose sugar from carbon dioxide and water.

SUBSTITUTES FOR PHOTOSYNTHESIS

Parasitic and saprophytic plants have no chlorophyll; but they have no use for it because they live upon organic matter and so need not produce their own food. Certain other plants, lacking chlorophyll, are not parasites or saprophytes; they must, consequently, have a substitute for photosynthesis. Such plants are the sulphur bacteria which oxidize sulphur. It is often stated that this process is a substitute for respiration; but this is not the case, for energy is gained, not lost, and with the energy so gained sulphur bacteria are able to synthesize carbon compounds.

In respiration energy is released; when bacteria oxidize sulphur, energy is taken in. The process is, therefore, comparable to photosynthesis, but light is not concerned. The activity of sulphur bacteria is another type of life phenomenon which must have been the only one by means of which plants grew before protoplasm chanced to synthesize chlorophyll. By analogy, the reaction may be termed "chemosynthesis"; in photosynthesis, light furnishes the energy; here a chemical change furnishes it.

ARTIFICIAL PHOTOSYNTHESIS

The theory that only living organisms form organic compounds collapsed when Wöhler synthesized urea. This does not mean that photosynthesis, or any other process occurring in cells, is not vital in so far as it is associated with living matter, nor should it imply that the process is necessarily as simple as it has been pictured. It may well be that photosynthesis is a function of the entire cell. Such reasoning may be correct, but it does not solve the problem; a situation is not helped by complicating it. Attempts to duplicate photosynthesis in a test tube should certainly not be frowned upon; only by such efforts has science advanced.

Willstätter arrived at his theory of photosynthesis through his ability to reproduce in the laboratory certain probable steps in the natural synthesis of sugar. He found that colloidal solutions of chlorophyll *a* and *b* unite with carbon dioxide, but could not reproduce subsequent steps in the process.

Baudisch found that when a solution of potassium nitrite and methyl alcohol is exposed to ultraviolet light, the alcohol is oxidized to formaldehyde. The reaction is photochemical and cannot be accomplished without light.

The work of B. Moore and E. C. C. Baly has been referred to; both attempted to convert water and carbon dioxide into formaldehyde with the aid of a fine colloidal suspension as catalyst and artificial light of very short wave length as the energy.

The most recent work of Baly involves the use of nickel oxide as catalyst. He claims that carbohydrates are produced when the surface of pure nickel oxide is irradiated with white light in the presence of carbon dioxide and water. The first product formed is said to be "a complex carbohydrate similar to starch. This starch, when acted upon by diastase, gives simple sugars similar to those produced in the leaves of plants." The laboratory process, according to Baly, is similar in every way to that in the living plant, the only difference being that in the former nickel oxide is substituted for the chlorophyll of leaves. Baly believes that there are no rays in sunlight capable of promoting photosynthesis as a single reaction, and so considers that the photosynthesis of carbohydrates is achieved in two successive stages. The first stage is promoted by blue light; and the product of this process, when acted on by the red light in the sun's spectrum, gives carbohydrates. The sum of the two amounts of the light energy thus supplied is exactly equal to the total amount of the energy required for photosynthesis.

This last suggestion is a very interesting one in respect to the usual question on the relative values of various parts of the spectrum in photosynthesis.

Of all these experiments on artificial photosynthesis, it can be said that whereas none as yet is free from criticism, they are to be commended as attempts to imitate a natural process which, if successful and commercially practicable, will greatly augment the world's supply of food.

Botanists have attempted artificial organic synthesis in a somewhat different way. Molisch exposed extracted chlorophyll solutions to light, with luminous bacteria as indicators of free oxygen, and found that free oxygen is liberated. The absorption of carbon dioxide and the liberation of oxygen in the presence of extracted chlorophyll suggest artificial photosynthesis.

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CHAPTER XIII

RESPIRATION

The food synthesized in the leaves of plants is transferred to other tissues where it is converted into protoplasm, stored for later use, or utilized in *respiration.*

Organisms require energy in order to maintain life. They obtain this energy by breaking down substances, usually organic ones, through the process of respiration. In a limited sense, this process consists of the oxidation of carbon compounds with a consequent release of energy. It takes place only in the presence of respiratory enzymes. The compound generally acted upon is sugar, and the substances formed are carbon dioxide and water.

$$C_6H_{12}O_6 + 6O_2 + \text{enzyme} = 6CO_2 + 6H_2O + \text{energy}$$

Although respiration usually involves oxidation through the addition of oxygen, or the removal of hydrogen, energy may also be derived from the splitting of compounds without oxygen, as in alcoholic *fermentation* by yeast.

The importance of oxygen to life was shown by both Priestley and Lavoisier. Lavoisier demonstrated that respiration and combustion are essentially the same, so far as energy yield is concerned; in both, oxygen unites with carbonaceous matter, liberating heat and carbon dioxide.

Respiration is the reverse of photosynthesis. In photosynthesis, as pointed out in Chapter XII, energy is stored in the production of sugar, through the union of carbon dioxide and water. In respiration, on the other hand, stored energy is liberated through the utilization of atmospheric oxygen in the decomposition of sugar. These and other contrasting facts pertaining to respiration and photosynthesis are fundamental. They may, therefore, be tabulated:

Photosynthesis	Respiration
$6CO_2 + 6H_2O \longrightarrow C_6H_{12}O_6 + 6O_2$	$C_6H_{12}O_6 + 6O_2 \longrightarrow CO_2 + H_2O$
Stores energy	Releases energy
Synthesizes sugar	Decomposes sugar
Carbon dioxide used	Carbon dioxide given off
Oxygen given off	Oxygen used
Takes place only in cells containing chlorophyll	Takes place in every living cell
Takes place only in the presence of light	Takes place at all times
Increases weight of plant	Decreases weight of plant

Respiration should also be distinguished from *digestion.* Food is stored as carbohydrate, fat, or protein, and later converted into sugar, which, being soluble, is suitable for translocation. The process of reducing higher foods to simpler and more soluble ones is *digestion.* Respiration and digestion are to be distinguished not only by what they accomplish, but also by the fact that digestion, contrary to respiration, uses energy.

Breathing is likewise not to be confused with respiration; it is simply the mechanical process of inhaling air, which, in higher animals, is done with the aid of lungs. Respiration is the subsequent utilization of the oxygen of the air in the breaking down of organic substances with a consequent release of energy.

Ever since Lavoisier demonstrated that carbon dioxide is given off in breathing, this gas has been regarded as poisonous; it, as well as the absence of oxygen, was thought to be the cause of asphyxiation. Today it is known that oxygen combined with 5 to 8 per cent of carbon dioxide is the ideal gas mixture for resuscitation from suffocation resulting from near-drowning or other accidents. To give the patient ample oxygen is not sufficient. The circulation of the blood and the tonus of the muscles must be reestablished. Carbon dioxide accomplishes this. Although no comparable studies have been carried out on plants, it may yet be true that a certain minimum of carbon dioxide is necessary for proper respiration in all living cells.

OXIDATION AND REDUCTION

It has been customary to distinguish respiration from certain other forms of oxidation, such as oxidation-reduction equilibria, by stating that the former is an irreversible change whereas the latter, as the term equilibrium implies, is reversible. By a perfectly reversible chemical reaction is meant one that requires the application of the same amount of energy to reverse it as that yielded by the original reaction. Oxidation-reduction systems may be completely reversible, partially reversible, or irreversible. The respiratory mechanism encompasses within its chain of reactions numerous oxidation-reduction equilibria. As these latter are an important part of any present-day respiratory scheme, they must be considered.

Formerly, oxidation meant simply the addition of oxygen, but now the reaction has come to mean a transfer of electrons. This may be accomplished by the addition of oxygen or by the loss of hydrogen. The reverse process, involving the release of oxygen, the gain of hydrogen, or an electron transfer in the opposite direction, is *reduction.* The numerous ways in which oxidation and reduction may take place through the addition of oxygen or loss of hydrogen are suggested in the following list of equations, where *A* is the substance oxidized and *B* the substance reduced:

$$\begin{array}{llllll}
A + \mathrm{O} & = A\mathrm{O} & & AH_2 + B\mathrm{O} & = A + B + H_2O \\
AH_2 + \mathrm{O} & = A + H_2O & & AH_2 + B & = A + BH_2 \\
A + B\mathrm{O} & = A\mathrm{O} + B & & A + H_2O + B & = A\mathrm{O} + BH_2
\end{array}$$

The oxidation of one substance always involves the reduction of another. Thus, in respiration, $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$; the sugar is oxidized and the oxygen is reduced. In assimilation, $6CO_2 + 6H_2O = C_6H_{12}O_6 + 6O_2$; the carbon is reduced and the oxygen oxidized. If oxidation and reduction processes are analyzed more carefully, it is seen that what actually happens is an increase in positive valence with oxidation, and a decrease in positive valence, or increase in negative valence, with reduction. This is to be understood as involving only a change in the number of valence electrons. Reduction involves an increase in electrons and a decrease in positive valence, whereas oxidation is the reverse; thus:

	Valence	Compound	Hydroxide or Acid
← reduction oxidation →	$+5$	N_2O_5	HNO_3
	$+3$	N_2O_3	HNO_2
	$+1$	N_2O	HNO
	-1	NH	NH_2OH
	-3	NH_3	NH_4OH

When iron in the ferric condition (e.g., as $FeCl_3$) is reduced to the ferrous condition ($FeCl_2$), the metal gains a negative charge resulting in a decrease in the number of positive charges, thus:

$$Fe^{+++} + H = Fe^{++} + H^{+}$$

As the ion, Fe^{++}, is capable of losing an electron, and so gaining a positive charge, it is regarded as a reducing agent or *reductant*; and as Fe^{+++} is capable of gaining an electron, it is an oxidizing agent or *oxidant*. The change from one to the other is a reversible reaction, and may be expressed thus:

$$Fe^{+++} + \text{electron} \rightleftharpoons Fe^{++}$$

DETERMINATION OF RESPIRATION

The methods devised for measuring respiration are numerous; they usually involve a determination of the amount of oxygen taken in or of carbon dioxide given off. This is best accomplished with the aid of a *respirometer* which, in its simplest form, consists of a series of bottles, one containing respiring material, such as moist seeds, into which carbon-dioxide-free air is allowed to pass, and another limewater into which the air from the seeds is directed (Fig. 42). The limewater removes, through precipitation of calcium carbonate, the carbon dioxide given off. The amount of carbonate is then determined by titration or

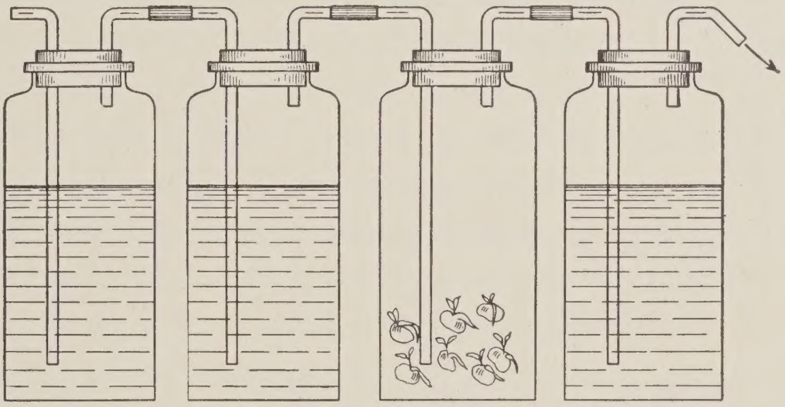

FIG. 42. Apparatus for demonstrating respiration. The first bottle on the left contains a dilute solution of sodium hydroxide; the second, lime water; the third, germinating seeds; and the fourth, lime water. A suction pump is attached at the right-hand end of the hose line. (After W. F. Ganong.)

otherwise. For quantitative work the carbon dioxide is collected in known quantities and analyzed (Fig. 43).

The rate of respiration, or *respiratory intensity*, is expressed in terms of the volume or weight of carbon dioxide formed or of oxygen consumed in relation to some quality affecting them or affected by them, such as leaf area or fresh or dry weight of the tissue.

ORGANIC SUBSTANCES UTILIZED IN RESPIRATION

Hexose sugars are the organic substances usually acted upon by respiratory enzymes. It is, however, very probable that other substances, such as fats or proteins, may serve as substrates. If respiration is more generally defined as that aggregate of reactions going on in living matter whereby energy is released, it will include a number of processes other than the oxidation of sugar.

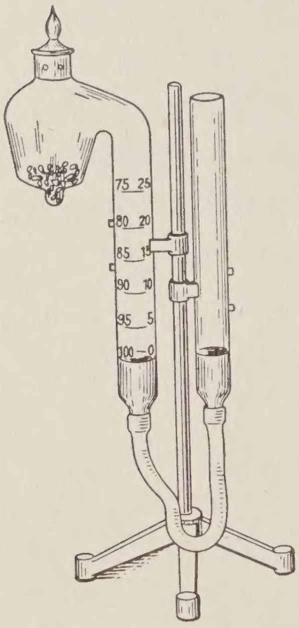

Fig. 43. Respirometer. (After W. F. Ganong.)

That organic substances are utilized in respiration is evident from the loss in weight of a respiring plant. This is not so obvious in plants where food is synthesized, or in animals where food is taken in rapidly enough to replenish the loss resulting from respiration; but, if food is neither synthesized nor absorbed, the loss in weight becomes very evident. An illustration of this is the weight of a seedling, before photosynthesis has taken place, compared with the weight of the seed from which it came; the dry weight of the seedling is less than that of the seed even though growth, with increase in size, has taken place.

After sugar, fat is generally presumed to be the source of energy which is later set free in respiration; but, if the fat is first changed to sugar, then the reactions simply involve digestion followed by respiration.

Fats and carbohydrates are the chief carriers of reserve energy. When they are exhausted, proteins may be used, though only in cases of extreme starvation.

FACTORS INFLUENCING RESPIRATION

Whereas the respiration of organisms ceases with death, it may be carried on by non-living matter. The very respiratory enzymes themselves will bring about the oxidation of sugar when removed from tissues. However, respiration always accompanies active life, even though it may not differentiate the living from the non-living.

Temperature, moisture, and injury all affect respiration. Increase in temperature produces an increase in the rate. Injury increases respiration by putting inner tissue into direct contact with the air, thus increasing the absorption of oxygen and the loss of carbon dioxide. Sunlight affects respiration; the rate is increased probably because of higher temperature and permeability changes.

The available supply of oxygen influences respiration, but only when the supply is greatly reduced. To injure the plant with excess oxygen, the concentration must be some twenty times that of the air. Respiration is slightly affected by the amount of carbon dioxide in the air.

Anesthetics, with which a plant, under natural conditions, would come into contact very rarely, stimulate respiration when applied in small doses but reduce it in larger doses. If, owing to high concentration of ether, respiration is lowered below a critical point, recovery does not take place.

Dormancy greatly reduces respiration. Though it may be so reduced as to become imperceptible, the process is considered to be still going on. This point of view is generally held by physiologists, yet it may be that viable seeds and spores of considerable age do not respire. Dormant protoplasm would thus be regarded as possessing all the potentialities of life, but not manifesting them until the proper environment is supplied.

The faster a plant grows, the greater is the amount of oxygen consumed and the greater the amount of carbon dioxide given off. Therefore, plants have a respiratory life curve which closely parallels their growth curve. This rule, however, does not hold rigidly; respiration may be high and yet growth slight.

The reason is not difficult to find; growth is dependent upon many factors, and respiration is but one of them.

RESPIRATORY QUOTIENT

The ratio of the volume of carbon dioxide given off to the volume of oxygen appropriated is expressed by the *respiratory quotient* and is written:

$$\frac{CO_2}{O_2}$$

Its expected value, when sugar is respired, is unity, as is shown by the equation:

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$$

but this is true only where such an equilibrium exists. If organic acids high in oxygen are used in the process, the respiratory quotient will be greater than unity; e.g., that of oxalic acid would be 4.0. If fats, which are poor in oxygen, are broken down, the respiratory quotient will be less than unity. The respiratory quotient of germinating seeds is less than unity.

Numerous factors influence the respiratory quotient; thus, some of the oxygen taken in may be used for other purposes and some of the carbon dioxide given off may come from sources other than respiration. Anaerobic respiration, which will be discussed later, proceeds without free oxygen; its respiratory quotient will, therefore, be infinity because in the equation CO_2/O_2 the denominator is zero.

In general, factors which influence respiration affect the respiratory quotient.

ANAEROBIC RESPIRATION

Respiration in which oxygen is used is said to be *aerobic*, but there are so-called respiratory processes in which oxygen is not consumed. These forms of energy release are known as *anaerobic respiration* or *fermentation.* When sugar ferments, carbon dioxide and ethyl alcohol result:

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH$$

Yeast and certain bacteria live by anaerobic respiration, but yeast may live aerobically as well.

When yeast is added to a sugar solution, bubbles of carbon dioxide soon appear, and the presence of alcohol may be detected. After a time, the rise of bubbles ceases, which is due either to decomposition of all the sugar by the yeast, or to narcosis produced by the alcohol. The activity of the yeast may be renewed through the addition of a slight amount of oxygen. If oxygen has been fully excluded during the fermentation, activity ceases even though food is still present. These facts give strong support to the contention that oxygen respiration occurs to some extent even in anaerobic organisms.

Most organisms which can live in an oxygen-free environment respire aerobically when oxygen is present and when the substances necessary for anaerobic respiration are not present. Yeast forms no alcohol and respires aerobically, forming carbon dioxide and water, when grown in cultures where peptone or lactose is the source of the needed carbon. If oxygen is withheld in such a case, the yeast dies. But if cane sugar is substituted for milk sugar, then alcohol is formed, i.e., the yeast has material for producing alcohol. It does so, and respires anaerobically whether oxygen is present in abundance or not. Only the hexoses, and the higher sugars which can be split into hexoses, are usually capable of being fermented. Among isomeric hexoses those closely related in structure, such as glucose and mannose, are fermented at nearly the same rate, whereas another but less similar isomer, galactose, is not fermented so readily. This means that certain lowly organisms which carry on fermentation have the remarkable capacity to distinguish between substances that are very closely related, so closely as to be identical in chemical properties, differing only in certain physical or optical qualities. The 3, 6, and 9 carbon-atom sugars, i.e., the tri-, hex-, and nonoses are fermentable; but the di-, tri-, and polysaccharides must be hydrolyzed into the 3, 6, or 9 carbon sugars before fermentation can take place. In certain instances it is only those sugars which rotate polarized light to the right, e.g., *d*-glucose, that are fermentable.

The remarkable capacity of organisms to choose from among very closely related substances was discovered by Pasteur (page 238).

As sugar could not be fermented without the aid of living protoplasm, men of vitalistic beliefs assumed that it was the living substance itself which accomplished the change. E. Traube, in 1858, suggested that a specific enzyme was the cause of fermentation, and the part played by the living organism was simply to produce the enzyme. Half a century later, artificial fermentation was accomplished. Yeast cells were killed with ether, yet their power of fermentation remained unaffected. An enzyme, therefore, is present which is not dependent upon life for its activity. The enzyme was named *zymase*. Today the term is used collectively for all enzymes responsible for yeast fermentation; alcoholase is a specific extract therefrom.

Anaerobic respiration is common to all fungi, but alcoholic fermentation is limited to but a few of them. *Bacillus butylicus* is strongly anaerobic; if oxygen is excluded from it, butyl alcohol is formed, together with carbon dioxide and hydrogen.

So perfect is the fermentation carried on by organisms and so imperfect man's attempt at an artificial reproduction of it that the yeast cell is resorted to in all commercial processes of alcohol manufacture.

Fermentation is the initial process in a number of series of reactions in nature: thus, the formation of alcohol through the fermentation of sugars in fruit juices is followed by oxidation of the alcohol into acetic acid:

$$C_2H_5OH + O_2 = C_2H_4O_2 + H_2O$$

This is what happens when sweet cider has turned into "hard" cider and then into vinegar. The fermentation is accomplished by the yeast occurring on the surface of fruits; bacteria convert the alcohol into acetic acid. The yeast works anaerobically, but the bacteria work aerobically, as oxygen is needed for further decomposition. Under suitable conditions, the acetic acid may be further decomposed with the formation of carbon dioxide.

FORMS OF RESPIRATION

In aerobic respiration,

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$$

and in anaerobic respiration or fermentation,

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH$$

there is a liberation of carbon dioxide, whereas in the oxidation of the alcohol formed by fermentation,

$$C_2H_5OH + O_2 = C_2H_4O_2 + H_2O$$

there is oxygen consumption but no liberation of carbon dioxide. What, then, is the criterion of respiration, oxygen consumption, carbon dioxide production, energy release, or, possibly, the nature of the substratum acted upon?

In general, plant physiologists regard the elimination of carbon dioxide as the chief characteristic of respiration; therefore, the fermentation of sugar by yeast is anaerobic respiration whereas the further conversion of the alcohol into acetic acid is not respiration because no carbon dioxide is formed. Animal physiologists regard oxygen utilization as the essential feature of respiration; therefore, fermentation is not a respiratory process but alcohol oxidation is. As long as the processes remain clear, the apparent contradiction is not serious, for the difficulty is primarily a matter of definition.

The chief function of respiration is the release of energy. As fermentation accomplishes this, it is a substitute for aerobic respiration. Certain bacteria have still another substitute for respiration; they are able to use the energy released in the oxidation of inorganic compounds containing sulphur and nitrogen. Oxygen is consumed, but the compound acted upon contains no carbon. *Beggiatoa* is the best known of the sulphur bacteria and occurs in spring waters containing hydrogen sulfide, which it oxidizes:

$$H_2S + O = S + H_2O$$

Sulphur is found in granules within the bacteria. Given a substratum of organic matter only, *Beggiatoa* dies off rapidly, unless supplied also with water containing hydrogen sulphide.

Certain other bacteria use different inorganic substances in respiration. The so-called *iron bacteria* are considered to be capable of converting ferrous into ferric oxide.

Some soil bacteria use compounds other than carbohydrates for their respiratory substrates, and the transformations proceed without oxygen. Putrefaction involves the decomposition of complex nitrogen-containing compounds into simpler ones. Ammonia is a common product of this process and is converted into nitrites and nitrates by special bacteria. The reactions are part of the nitrogen cycle (page 167). Such processes are forms of anaerobic respiration involving compounds other than carbohydrates.

There are many examples of energy-releasing reactions in organisms not involving decomposition by oxidation; thus, whereas the decomposition of formic acid into carbon dioxide and water, involves oxygen consumption:

$$HCOOH + O \rightarrow H_2O + CO_2$$

the production of carbon dioxide and hydrogen from formic acid requires no oxygen:

$$HCOOH \rightarrow H_2 + CO_2$$

In both cases an enzyme is the catalyst; in the latter reaction, the enzyme is dehydrogenase, produced by bacteria. Both reactions release energy, but whether or not they are forms of respiration depends upon one's interpretation of this process.

If any process which is a source of energy for the organism is thought of as respiration, even though the release of energy involves no oxygen consumption, as in fermentation, nor the production of carbon dioxide, as in the oxidation of hydrogen sulphide, then the rather special case of so-called *urea fermentation* can be looked upon as respiration.

$$CO(NH_2)_2 + H_2O \rightleftharpoons CO_2 + 2NH_3$$

No oxygen is consumed nor carbon dioxide liberated in the decomposition of urea by soil bacteria; yet the significance of the reaction for the organism is the same as in respiration, namely, the release of energy.

Returning now to the more rigid point of view held by the physiological chemists, the foregoing decomposition of urea is simple hydrolysis and not respiration. No restrictions are placed on the nature of the substrate acted upon nor on the end products resulting therefrom. The substrate may be any *metabolite*, not only carbohydrate, and the end products may be CO_2, H_2O,

H_2SO_4, NH_3, or urea. The only necessary condition is that there shall be oxygen consumption. This and this alone is respiration for the physiological chemist. To him, all forms of non-respiratory reactions releasing energy are fermentation.

THE MECHANISM OF RESPIRATION

Pfeffer in Germany, then Kostychev and Palladin in Russia, and finally Blackman in England, developed and elaborated a theory of plant respiration which laid the foundation for modern theories. Pfeffer assumed that aerobic respiration proceeded in two stages; first, the fermentation or anaerobic splitting of sugar into ethyl alcohol and carbon dioxide, and then the oxidation of the ethyl alcohol into carbon dioxide and water. The theory as modified may be diagrammed thus:

Reserve substances → normal hexoses → activated hexoses →

$$\begin{tikzcd} & (O_2 \text{ present})\ CO_2 + H_2O \\ \text{3-carbon compounds} \arrow[ur] \arrow[dr] & \\ & (O_2 \text{ absent})\ CO_2 + C_2H_5OH \end{tikzcd}$$

Bennet-Clark, W. O. James, and others have suggested the following path: starch→ sucrose→ γ fructose→ 3-carbon compounds → pyruvic acid → acetaldehyde + CO_2 → acetic acid → succinic acid → fumaric acid → oxaloacetic acid → oxalic acid → CO_2 + H_2O. Here is the first indication of what will be strikingly evident later on, namely, that the chain of reactions suggested by Pfeffer and other pioneer workers on plant respiration and simply expressed in the equation, $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$, represents but the barest outline of respiration.

That plants and animals contain substances which function as oxidizing agents has been known since the middle of the last century. These agents are called *oxidizing enzymes, ferments*, or *oxidases*. Associated with them are the *peroxidases*, which activate the oxygen of hydrogen peroxide, and *catalases*, which decompose hydrogen peroxide into water and oxygen. The darkening of the cut surface of fruits and vegetables is due to the activity of oxidases.

Warburg attributed oxidation in respiration to a single enzyme or *Atmungsferment*. Wieland, viewing the problem more broadly, held that several respiratory enzymes are responsible, one among them being *dehydrogenase.* He postulated that the loss of hydrogen by the organic substrate was the essential feature of respiration rather than the addition of oxygen. The transfer of the hydrogen from the organic substrate to the oxygen of the air was accomplished through the enzymatic activity of *dehydrogenases.* The activated hydrogen presumably unites with atmospheric oxygen to form hydrogen peroxide. The substrate is, therefore, oxidized, through the loss of hydrogen, and the atmospheric oxygen reduced.

Here the matter rested with no general agreement until Keilin discovered that all cells (anaerobes excepted) contain a pigment which he called *cytochrome.* He postulated that cytochrome acts as an acceptor of the hydrogen from the substrate which has been activated by dehydrogenase, as postulated by Wieland. The reduced cytochrome is then reoxidized by oxygen activated by the Warburg respiratory enzyme. The regenerated cytochrome functions again as the hydrogen carrier. Cytochrome is thus a third and intermediary enzyme. The whole chain of reactions may be diagrammed as follows:

$$\text{Organic substrate} + \text{Wieland enzyme} + \text{cytochrome} \rightarrow \text{CytH}_2 + \text{Warburg enzyme} + O_2 \rightarrow \text{Cyt} + H_2O$$

The foregoing outline of the respiratory mechanism did not long stand unquestioned, for Warburg soon discovered another respiratory enzyme, a yellow pigment, not identical with any so far mentioned; its function is probably that of a carrier.

These discoveries, though not final, have opened up a much wider horizon and emphasized again how little is told by the simple chemical equation, $C_6H_{12}O_6 + O_2 = CO_2 + H_2O$.

A recapitulation, following M. Dixon, will help clarify these recent contributions to theories of respiration.

The cell contains catalytic mechanisms which permit it to utilize molecular oxygen for the oxidation of organic metabolites. This is respiration.

One of the earliest suggestions, revived by Warburg, attributed respiration to the activation of oxygen. An iron compound was assumed to be the respiratory catalyst.

The next suggestion came from Wieland who thought that hydrogen was the element activated. This was proved true by the discovery of a series of intracellular enzymes, the *dehydrogenases*, with precisely this function.

The fact that certain of the metabolites, even when activated by their dehydrogenases, do not react with molecular oxygen led to the third contribution, first clearly stated by Szent-Györgyi, namely, that both oxygen activation and hydrogen activation take place, and in such a way that active hydrogen reacts with active oxygen.

The last of the chain of events was contributed by Keilin. He discovered the necessary go-between or *carrier*, without which the active hydrogen cannot react with the active oxygen. This hydrogen carrier is *cytochrome*.

The idea of a respiratory carrier was first put forward by F. G. Hopkins, who suggested that this function was fulfilled by glutathione. Although this compound may account for a small part of respiration, Keilin's cytochrome and the more recently discovered *flavoprotein*, or yellow pigmented enzyme, of Warburg, are now recognized as the important carriers of respiration, accounting for practically the whole of respiration in yeast and certain bacteria.

Again there is evidence that Willstätter's hypothesis advanced years ago is true, that enzymes consist of two components, a colloidal carrier and an active group (page 200). The carrier is a protein and is specific. The yellow respiratory ferment is a union of a nitrogen-containing compound, a sugar group, and a phosphoric acid. The nitrogen compound with the sugar group may occur free as the pigment, *flavin* (see also page 201).

In the course of the foregoing work, particularly the work of Warburg, the importance of iron in respiration has become evident. This element occurs both in the blood and in the cell. In the blood, iron serves as a carrier of oxygen from the lungs to the tissues. The iron enters the lungs as a divalent metal where it is converted to trivalent iron through the addition of another molecule of oxygen which it later liberates in the cells of tissues as free unactivated oxygen; in so doing, it is again reduced from the ferric to the ferrous condition. In cells, the respiratory iron is not a carrier, but a catalyst. It is bound to the respiratory ferment.

One significant fact which has come out of recent research is that respiration may take more than one path; thus Elliot and Dixon showed that some 10 to 15 per cent of respiratory substances are consumed by mechanisms other than those involving cytochrome; some react directly with oxygen.

The absence of any reference to carbon dioxide formation in the foregoing respiratory mechanism of Wieland, Keilin, and Warburg is unexpected, as this gas is regarded by the biologist as an unfailing indication of respiration. Carbon dioxide is produced, however, in various subsidiary oxidations; for example:

$$HCOOH + O \rightarrow CO_2 + H_2O$$

One final word should be said in regard to the general applicability of any scheme of respiration. That the course of reactions may proceed in one of several directions has been stated, and this may mean that the chain of events in plant respiration differs from that in animals. Peroxidase is known to be universally present in plant cells, and cytochrome is believed to occur in all cells, excepting anaerobes, but not all the known respiratory enzymes may be of general distribution. Simply viewed, respiration is essentially the same in all organisms; oxygen is taken in, sugars decomposed, and carbon dioxide and water produced, but the paths taken may be different.

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CHAPTER XIV

THE CARBON AND NITROGEN CYCLES

Autophytic plants synthesize their own food, and in so doing accomplish one of the most important of physiological processes, the binding of carbon and nitrogen. This constant formation of organic compounds would soon exhaust the available supply of carbon and nitrogen on earth were there not some means for releasing bound carbon and nitrogen and making them again available for synthesis. Such a means exists; it is part of the *carbon* and *nitrogen cycles*.

THE CARBON CYCLE

As in all cycles, a start can be made at any point, and in the carbon cycle this point may conveniently be the carbon dioxide of the air. Plants utilize this gas with water to synthesize sugars by means of photosynthesis. From these sugars higher foods are formed through the agency of catalysts in the tissues where the foods are used or stored. Sooner or later the autophytic plants die or are consumed by animals which give off waste products, and in their turn die. With death, decay is brought about by bacteria and fungi. The autophytic plants which bind carbon, the animals which digest and reconvert foods, the fungi which rot organic matter, all respire, and thus break down sugars with the release of carbon dioxide. Thus, through respiration, the carbon bound by photosynthesis is returned to the atmosphere.

THE NITROGEN CYCLE

Organic nitrogen follows a cyclic course which is in part within the plant and in part within the soil. This continuous path is known as the *nitrogen cycle*. The plant draws its nitrogen from the soil, incorporating it with carbon-containing compounds to form amino acids, then grouping them into proteins which are used by the plant as food, reserve material, or constituents of protoplasm. With the death of the plant, the nitrogenous material again reaches the soil; or the plant, being eaten by animals, ultimately reaches the soil as waste or dead organic matter. These nitrogen-containing substances are acted upon by bacteria and fungi; *decay, putrefaction*, or *ammonification* results. Ammonia is formed and carbon dioxide set free, the liberation of carbon dioxide indicating respiration.

Saprophytic bacteria primarily, but also other fungi, and a varied microfauna decompose organic matter in the soil. Ammonification is the first step in the decomposition of nitrogenous matter. It involves the formation of ammonia, water, and carbon dioxide. The ammonia goes into solution and the hydroxide thus formed reacts with acid ions in the soil solution, forming ammonium salts such as acid ammonium carbonate and ammonium nitrate.

The conversion of organic nitrogen into ammonia is accomplished only by microorganisms, and the reaction is specific, for only certain organisms, such as *Bacillus vulgaris*, are capable of bringing about the change.

The next step in the nitrogen cycle is the conversion of ammonium nitrogen into nitrites. This transformation is also specific, and is brought about by a microorganism known as *Nitrosomonas*.

The final step in the production of soil nitrates from waste organic matter is accomplished by the organism, *Nitrobacter*; it oxidizes nitrites into nitrates. Both these nitrogen-transforming processes are carried out by anaerobic microorganisms, which are able to utilize the energy liberated from these reactions for the binding of carbon, that is to say, they do not require organic food in the same way as do the parasitic and saprophytic bacteria, but synthesize their own food and thus become autotrophic organisms (see also page 149).

The question whether or not the green plant can utilize nitrogen in forms other than nitrate has long been answered in the negative. This conclusion was based on early work by Boussingault, who emphasized the importance of nitric acid united to a base as a nutrient for the green plant. His work was significant because it contradicted the opinion of Liebig prevailing at the time (1840-60) that ammonia was the chief source of nitrogen for the plant, a conclusion based on the fact that fertilizing with ammonium salts was observed to produce excellent results. Liebig failed to realize, and at the time could not know, that ammonia is converted into nitrates in the soil. Molisch then showed that nitrites, which precede the formation of nitrates in the soil, are toxic to plants when in high concentrations. On the whole, the foregoing views are still held, but more careful work has shown that plants are able to utilize both ammonium salts and nitrites, though less successfully than nitrates. The availability of soil nitrogen is partly a question of toxicity. Ammonium nitrite is mildly poisonous; so also ammonium carbonate, apparently because of its alkaline reaction. Other ammonium salts, especially the sulphate, can be very useful to plants, particularly when nitrates are low, as on woodland soil.

The possibility of the green plant assimilating nitrogen from organic compounds has been investigated, but with very few positive results, and in no case was the gain in weight significant. Among organic nitrogenous materials believed to be of a small advantage to green plants are glycocoll, leucine, asparagin, amines, peptone, urea, and dried blood.

The question of other possible sources of nitrogen for the plant is not only of theoretical interest but also of practical value, as the quantity of nitrates in most soils is small; indeed, plants have always to contend with a scarcity of nitrogen.

Nitrogen Fixation. Any process whereby free, gaseous nitrogen is united or bound to other elements to form a compound is known as *nitrogen fixation.* Some organisms, particularly certain soil bacteria, fix nitrogen in the course of their ordinary life; it is also carried out artificially on a large scale.

When lightning traverses the air, the free molecular nitrogen is oxidized into nitric and nitrous acids, which are brought down in solution by rain. The fixed nitrogen so produced is not more than 1 or 2 per cent of the plant's needs and cannot, therefore, add materially to the total requirement of the plant. Commercially, however, the fixation of atmospheric nitrogen has become an industry of tremendous importance. It is accomplished either by passing a spark through the air, or by allowing nitrogen and hydrogen to pass again and again through a metal receptacle under a pressure of 200 atmospheres at a temperature of 500°C with iron or other metal as catalyst. The ammonia gas formed is directed into water for absorption. The artificial fixation of atmospheric nitrogen throws no light on the method used by organisms. There is no conclusive evidence that atmospheric nitrogen is used by green plants, but its fixation by certain non-green plants is a well-established fact.

Many soil microorganisms are active in the fixation of atmospheric nitrogen. The aerobic *Azotobacter chroococcum* and the anaerobic *Clostridium pasteurianum* are the chief nitrogen-fixing bacteria of the soil. In the usual process of decay, some nitrogen is lost into the atmosphere; this is recaptured by soil bacteria. Other bacteria and fungi, in particular the molds, *Aspergillus* and *Penicillium*, may also take part in nitrogen fixation.

The custom of dividing crop plants into the two groups, legumes and non-legumes, is one of long standing and well founded, for it has been known since ancient times that peas, beans, vetches, clovers, alfalfa, lupines, and related plants enrich the soil, whereas a continuous growth of cereal and other non-legumes leads to a more or less rapid decline in soil productivity. In the course of the last century, a more complete knowledge of plant nutrition was attained, and it was found that the difference between legumes and non-legumes is due to the manner in which these two groups of plants obtain their nitrogen. All ordinary legumes have associated with them bacteria which fix atmospheric nitrogen. Non-legumes lack such bacteria and must, therefore, acquire their nitrogen from the soil instead. If a soil is rich in nitrogen, the legumes, too, may draw on this source.

Legumes owe their remarkable ability to use atmospheric nitrogen to certain bacteria which live in the root tissues. These bacteria gather the free nitrogen and transform it into compounds which are taken up by the host plant. The bacteria live in nodules on the roots of the legumes (Fig. 44). The number of nodules which may develop on a plant root varies from a few to several thousand. Some native legumes, of which the most common are the locust, *Robinia,* the Kentucky coffee tree, *Gymnocladus dioica,* and wild senna, *Cassia marilandica,* apparently do not produce nodules. There are, on the other hand, some non-leguminous plants which possess root nodules very similar to those of legumes; those so far discovered are the alder, *Alnus,* Jersey tea, *Ceanothus americanus,* Buffalo berry, *Shepherdia argentea,* and certain cycads.

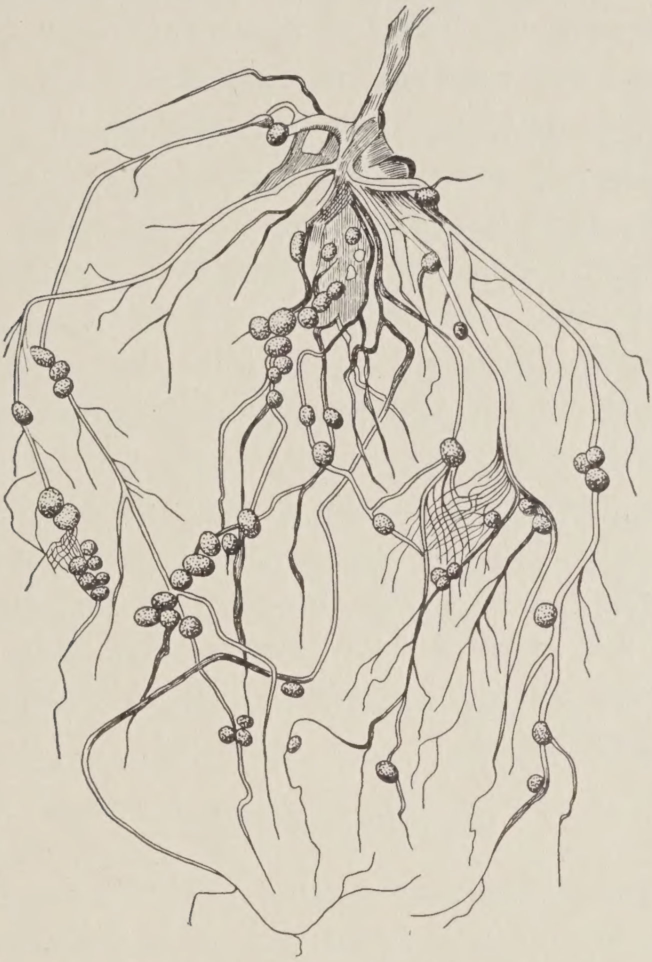

FIG. 44. Nodules on the roots of a legume.

It may be readily shown experimentally that the members of the pea family possess some unusual power to obtain nitrogen from soil poor in nitrogenous compounds. Neither legumes nor cereals grow well in pure quartz sand when it is free from nitrogen-containing compounds and has been sterilized by heating as well; but, if a small quantity of unheated soil is added to the nitrate-free sand, the lupins show a tremendous increase in weight whereas the oats remain unaffected. If the added soil is first heated to 70°C, no such increase occurs; thus it is evident that the unsterilized soil carried with it living material of value to the legume and not to the cereal.

When the nodules on the roots of legumes are cut open and examined under the microscope they are seen to contain colonies of bacteria. They were formerly called *Bacterium radicicola*, but now go by the name of *Pseudomonas radicicola.*

The swollen appearance of the root would lead one to believe that the bacteria are parasites, but the evident benefit to the green plant suggests more that the plant is parasitic on the bacteria. Actually, there is an exchange of food stuffs; carbohydrate is given in return for bound nitrogen. Such a mutually beneficial association is termed *commensural symbiosis* (see page 275).

The soils of the western United States are well supplied with the proper bacteria for the natural inoculation of alfalfa; but in the eastern part of the country, where soil conditions are on the whole less favorable to the growth of nitrogen-fixing bacteria, it is nearly always necessary to supply them at the time of the first sowing of this crop. Inoculation may be accomplished by scattering soil from a successful alfalfa field or soil from around the roots of sweet-clover plants; or cultures of the bacteria may be distributed.

DENITRIFICATION

While the foregoing constructive collection of nitrogen from waste organic matter and from the air is taking place, certain other soil bacteria are active in releasing nitrogen. The bound nitrogen in nitrates is set free as gaseous oxides of nitrogen or as free nitrogen; this is *denitrification.* The bacteria responsible for it occur mostly in unrotted organic matter and in soil poorly aerated. The loss is not great in fertile and properly cultivated soils.

CHAPTER XV

THE MOVEMENT AND STORAGE OF FOODS

TRANSLOCATION

Foods manufactured in the leaves of plants must be moved or *translocated* to tissues where they are to be used or stored. The problem of food transfer in plants involves, first, the question of the kinds of foods that are shipped, and then how the translocation is accomplished.

It would not seem to be a great task to ascertain which foods are transferred in plants, but the difficulty lies in the fact that it is impossible to be sure which of the foods found in cells are on the way to other cells and which have been synthesized in the cell where they are found. Finding coal in a ship's hold would not in itself tell one whether it is destined for a foreign port or for the ship's own use.

The question of the kind of food translocated in plants may be approached indirectly. The first permanent organic matter synthesized in leaves is sugar or starch. The higher foods are built from these. Sugar offers more advantages for translocation than any other kind of food; it is readily soluble in water; its molecules are of comparatively small size permitting ready diffusion through protoplasmic membranes; and it is the form in which cells use organic matter for respiration. Although there is some evidence that starches, proteins, and even fats are moved in the plant, it seems likely that sugar is transferred in greater quantity than any other organic substance.

Before the mechanism of translocation can be considered, the path that food takes must be definitely determined. It is generally stated that the phloem sieve tubes are the channels through which food passes from the leaves to all parts of the plant. If protoplasm is the carrier, then the phloem vessels satisfy all conditions, for not only are they alive, but there is a continuous connection of protoplasm between them in the form of strands which pass through perforations in the sieve plates. But sieve tubes are only the main arteries; food goes into every cell. Unless protoplasmic strands connect all living cells, then food transference from cell to cell must be by means other than those involving protoplasmic movement.

Diffusion will account for movement through both living cells and dead cells. It is only necessary that there be a concentration gradient. As sugar is produced in the leaf, the concentration there will be higher than in the adjoining cells; but the gradient must be maintained. The translocation of food continues through the night, so that by early morning it is fairly complete. This means that the transfer of sugar continues after the concentration gradient has reversed itself. Over a twenty-four hour period, the sugar content in cells of the stem where sugar is stored is higher than in the leaf. Mason and Haskell found a sugar concentration of 1.5 per cent in the leaf, 3 per cent in the phloem, and 0.5 per cent in the xylem. These facts do not necessarily invalidate the diffusion hypothesis of food transport, but they must be met.

Those determinations which established that the leaf is free of food by early morning were made in respect to starch. If sugar entering storage cells is immediately converted into starch, then the concentration of sugar there is kept virtually at zero; consequently, movement of sugar may continue until the concentration is near zero everywhere.

There is another possible situation which will explain why the findings of Mason and Haskell appear to stand in opposition to sugar transport by diffusion. A chemical analysis of sugar content will give the total amount present without regard to its condition. If a determination of concentration is to be significant in diffusion, it must rest on the same basis as diffusion-effective conditions. For example, colloidally bound sugar will appear in an analysis, yet it is not free for diffusion.

There is also the question of rate: the rate of movement of sugar in tissues as measured in terms of concentration at various points is much faster than the diffusion rate of sugar molecules in water; because of this, certain accessory forces have been postulated, such as a "Druckstrom." Were there a pressure current, it would be unidirectional; and this is in conflict with the observations of Mason and Phillis that carbohydrate and protein travel simultaneously in reverse directions through the phloem.

Still another question to be considered is the permeability of the cell membrane. In the discussion of osmosis, sugar was chosen as the chief osmotically active substance in the living cell, and the protoplasmic membrane was assumed to be impermeable to it. If sugars maintain cell turgor, they obviously cannot readily penetrate the cell membrane. An escape from this difficulty is found in the suggestion that the larger molecules of the disaccharides might maintain turgor whereas the smaller molecules of hexose sugars are translocated. After emphasis is thus laid on the sugars as the inferred guardians of cell turgor, it is disconcerting to find by actual measurement that it is the electrolytes which apparently are responsible for cell turgor, for these have smaller molecules than the sugars and should, therefore, pass still more readily from cell to cell.

Although the movement of food is generally assumed to be by way of living tissue, a sugar-maple tree is tapped beyond the cambium ring into wood consisting of dead xylem elements. It is out of the xylem that sugar in solution comes. The sugar is translocated from the leaf through the phloem and is stored in the phloem of the tree trunk. During late fall and winter, sugar penetrates the xylem by diffusion or internal "bleeding" under pressure. When the xylem is tapped in the spring there is as yet no transpiration stream and the movement of sugar is not unidirectional, i.e., up the tree only. Tests made of sap pressure in sugar maples at the time of tapping show this pressure to be in all directions. In general, the movement of food is not only downward from the leaves, but also upward, lateral, and around the stem. Lateral translocation takes place mostly in the pith or medullary rays through living cells with no special features adapting them to conduction as in the case of phloem.

From this conflicting data no definite conclusion can be drawn as to the mechanism of food transfer. As the translocation of organic substances takes place in living cells, protoplasmic movements may play a part. Diffusion may be a factor, but it is too slow to account for rapid movement of food. It will, however, explain food transfer in dead cells where this occurs. The movement of food appears to take place under pressure; consequently, those same forces responsible for "bleeding" (page 81) may be involved.

In addition to the movement of water and salts through xylem, and the movement of organic matter in phloem and parenchyma, there are a number of specialized conducting systems such as latex tubes and resin ducts. The conducting systems through which latex (page 205) flows differ in type, but all latex cells are living, the protoplasm forming a very thin layer in them.

The movement of food from place to place in the plant accomplishes two purposes: cells are supplied with their nutritional needs and reserve materials are stored.

STORAGE

The problem of food storage, broadly viewed, involves movement to the place of storage, the place where stored, conversion into a form suitable for storage, and conversion or digestion into the original or other adequate form for translocation to growing tissues.

The movement of food to its place of storage probably occurs, as already suggested, in the form of sugar, for sugar is readily soluble, and is one of the first permanent products of photosynthesis.

Where stored food is built up from sugar and again broken down into sugar for translocation and respiration, storage can take place only in living cells in order that the necessary enzymes for the anabolic and catabolic processes shall be present. It is probable that all cells have the power to synthesize higher foods, as they do protoplasm.

Carbohydrates, proteins, and fats accumulate in large proportion in tissue known as *storage parenchyma.* This tissue is of wide distribution in the plant and consists of living, thin-walled cells which apparently have no other function than that of storage.

Storage tissues usually show no special modification, but in some cases stems and roots become highly modified; in certain instances the change may be so great as to make the organ quite unrecognizable. Little or no modification of storage tissue occurs in perennial woody plants, where the parenchyma, particularly the medullary rays, are the chief storage regions. Slight modification is found in certain herbaceous perennials which develop fleshy stems, as asparagus and rhubarb. The succulent stems of cacti and other plants of arid regions, such as *Agave* and *Sempervivum*, possess specialized water-storage tissue. Underground stems and certain roots are among the most highly specialized of food-storage tissues; such stems include tubers, bulbs, corms, stolons, and rhizomes; and the roots include the sweet potato, carrot, beet, and radish. Storage roots often assume massive proportions owing to the quantity of food stored therein. The tropical yam may be one to two feet long and six inches in diameter. Storage tissue is not always located in the same place in reference to the cambium ring: in the turnip (*Brassica*) it is wholly within the cambium ring; in the parsnip (*Pastinaca*) it is wholly without; in the carrot (*Daucus*) it is partly within and partly without.

Leaves serve as storage tissue only if they are to persist, for it is evident that food stored in a deciduous leaf will be of no use to the plant. Some food always remains in a fallen leaf, and fungi and bacteria find it. Typical storage leaves are those of cabbage and onion.

A flower rarely serves as storage tissue until it has ripened into a fruit, but there are certain familiar exceptions, such as the cauliflower and artichoke.

Practically all seeds contain stored food which serves the useful purpose of later nourishing the germinating embryo. Orchid seeds are a notable exception.

Fruits are outstanding examples of food-storage tissues. The wall of the ovary becomes highly modified in ripening. The fleshy edible portions of the peach, cherry, orange, and tomato are fructified ovary walls rich in stored food. The fleshy portion of the apple is the swollen receptacle of the flower.

The kinds of food stored include all organic compounds found in plants, chiefly sugar, starch, protein, and fat. Whereas some of each of these are usually found in all storage tissues, most often one or the other predominates, as does sugar in cane and beets, starch in potatoes, protein in grains, and fat or oil in certain seeds. Whether these serve solely the purpose of reserve energy or have other uses, cannot always be stated definitely. Among the carbohydrates, starch is the chief food stored, and sugar is next in abundance. Cellulose, particularly the hemicelluloses, may possibly serve as reserve food. From the point of view of efficiency, it is the fats and proteins which make the best form of reserve energy. Food stored as fat or protein occupies less space than sugar if the energy reserve is the same. There are other benefits; fats and proteins are more stable. Furthermore, the osmotic value of the cell sap must be maintained at a definite level; added sugar would upset the equilibrium.

All forms of organic food are stored in seeds and fruits, and many find economic value. The fleshy cotyledons of peas and beans are rich in starch. Grains have much protein, as in the *aleuron layer* of corn; and seeds such as those of coconut, cotton, and flax serve man with the fats and oils they contain. Sugar, water, and cellulose constitute the bulk of many fruits such as melons. Bananas are mostly starch, and oil makes up a large part of the fruit of the olive.

Fleshy fruits serve the plant in seed dispersal through the agency of fruit-eating animals, particularly birds.

Food stored in the fall is shipped in the spring; to accomplish this it must be broken down or digested into a form suitable for movement in the plant.

Insoluble starches, proteins of high molecular weight, and insoluble oils are not suitable forms for translocation. Sugar, on the other hand, offers all the advantages of food suitable for efficient transfer in the plant. The digestion of complex organic compounds is accomplished by enzymes in the cells where the food is stored.

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CHAPTER XVI

FOODS

CARBOHYDRATES

The chemistry of plant foods is no longer an integral part of an introduction to plant physiology. It has outgrown its parent. Therefore, only those properties of foods will be considered here which contribute to an understanding of the living plant.

The foods of plants are the same as the foods of animals. Inorganic substances are not foods. To be sure, if a plant containing chlorophyll is supplied with inorganic matter only, it will grow normally; but the salts supplied are not food; this is made by the plant. Food is organic material, for a plant as well as for an animal. Fertilizers are, therefore, not properly termed "plant foods." Sugars, starches, proteins, and fats are the real foods.

Botanists have generally assumed that the first food synthesized in plants is a hexose sugar, but evidence in favor of starch repeatedly occurs (page 145). If it is a hexose sugar, then through condensation sucrose and other disaccharides are quickly formed. Should the first food synthesized prove to be starch, then the sugars observed are the result of the action upon it of diastase or other enzymes. More complex foods are synthesized by special enzymes within the cells where the food is to be used or stored.

Sugars serve two primary purposes in plants. They are the most convenient form in which to transport food, from the leaf where made to the root where used, and they are the form to which stored organic foods are reduced for respiration.

By growing isolated roots as autonomous units (page 241) it has been shown that sucrose rather than glucose is required as the primary source of energy by some plants.

The starches and celluloses are other important plant polysaccharides. Starch serves well as storage material, in that it is poorly soluble in water and is non-fermentable until inverted into hexoses. Cellulose is the primary constituent of cell walls, and as such receives special consideration in a separate chapter.

The conversion of stored starch into other plant materials at the time of seed germination is illustrated in determinations made by Boussingault, a pioneer plant physiologist.

	SEEDS	SEEDLINGS
Starch and dextrin	74.	17
Sugar	0.	21
Oil	5.	3
Cellulose	6.	29
Protein	10.	20
Remainder	5.	10
	100	100

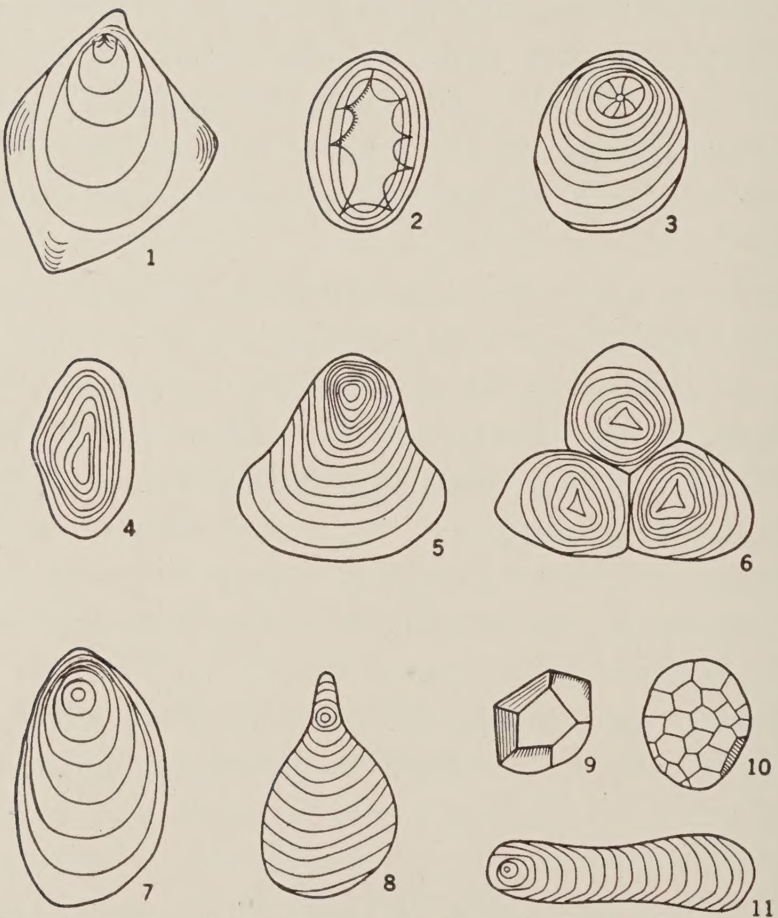

FIG. 45. Starch grains from eleven species of plants showing characteristic markings: 1, *Marantia;* 2, bean; 3, sago; 4, pea; 5, *Oxalis;* 6, *Smilax;* 7, potato; 8, canna; 9, oat; 10, corn; 11, hyacinth.

Cells active in forming starch usually do so at starch-forming centers known as *amyloplasts* and *pyrenoids*. The synthesis is accomplished by a catalyst, comparable to chlorophyll but colorless and not needing light as energy. The starch is secreted in layers about an initial deposit. This gives to the grain a characteristic stratification (Fig. 45) which has been used not only as a basis for starch classification but also for the classification of plants. The natural starch grain consists of at least two materials, starch proper, surrounded by a covering of a starch-cellulose complex. These two materials become evident when the grains are digested by diastase; the inner starch goes into solution, leaving a shell of cellulose.

A rather novel and unexpected function has been ascribed to starch grains by Haberlandt and Němec. They suggest that the position of the grains in the cell determines the direction of growth; that is to say, in assuming their position at the bottom of the cell because of gravity, the grains serve as *statoliths* or gravitational sense "organs" (Fig. 46). They may do so by stimulating the cell to auxin or other hormone production (page 212).

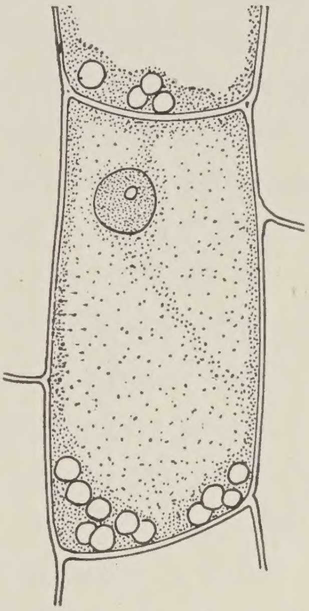

FIG. 46. Starch grains presumably serving as statoliths in a plant cell.

Certain other carbohydrates play a more important part in plant life than is ordinarily accredited to them. Among these are the *pentosans*, which include the vegetable gums and the mucilages. They are well characterized by the German word "Pflanzenschleim." The water-holding power of the pentosans suggests their use in the plant for water storage, as in the stems of cacti. It may also be true that the pentosans are important constituents of the substratum of living matter, for, like them, protoplasm is hydrophilic, elastic, and slimy.

ACCESSORY CARBOHYDRATES

Protection of the plant from bacterial decay and other harmful influences such as dehydration, has to do with its well-being, and so with its physiology. Two methods of accomplishing protection are the impregnation of the wood and the secretion of a resisting layer at the surface, consisting of substances, certain of which are carbohydrates.

Wood, in time, undergoes *lignification* through the deposition of *lignin* in the cellulose walls of the cell. *Suberin* impregnates the walls of cells in the bark, converting wood into cork. Resinous substances also impregnate wood and protect it. *Cutin* is deposited at the surface of epidermal cells, forming an outer coating relatively impermeable to water. The outer surface may be coated with wax instead of cutin, serving the same purpose.

The relationship between cellulose and its impregnating substances is not known, but it appears likely that the cellulose undergoes a chemical change or rearrangement, a new compound, such as *lignocellulose*, being formed.

PROTEINS

There is an old proverb which runs, "Four elements intimately mixed form all life." These four elements are carbon, hydrogen, oxygen, and nitrogen. They are the basic elements of proteins.

One frequently finds emphasis laid upon the proteins as the fundamental constituents of protoplasm. Thus Pauli says, "They alone display the specific properties of life"; and Jost adds, "Bodies composed only of carbon, oxygen, and hydrogen cannot be endowed with vitality." It is speculative to regard any one substance in protoplasm as more fundamental than another, yet it does appear that nitrogen-containing compounds serve to build tissue whereas the nitrogen-lacking ones are primarily nutritive.

A number of times it has been stated (page 173) that organic matter in the plant is transferred from cell to cell as sugar; the reasons for this view are the solubility, ease of penetration through the cell membranes, and the abundance of sugar in plant sap, particularly at the time of mass movement of food in late spring. However, if only sugar were transferred, then there would be no nitrogen, sulphur, or phosphorus, out of which to form proteins in those cells where these compounds are synthesized. This difficulty is met by the fact that nitrogen, sulphur, and phosphorus may occur as salts throughout the plant and are, therefore, available wherever needed. It may also be true that the amino acids, the "building stones" of the proteins, are moved from tissue to tissue, and are combined into proteins where needed.

The amino-acid requirements for plants seem to be quite similar to those of animals with some few exceptions, as shown by P. R. White working with isolated tomato roots (page 242).

The empirical formula of a protein, $NH_2—R—COOH$, indicates that there are two groups, the amino and carboxyl radicles, both capable of ionization, one forming the positive ion, NH_3^+, and the other the negative ion, COO^-. The relative number of ionized amino and carboxyl groups determines the properties of proteins, their charge, migration in an electric field (page 126), powers of adsorption, etc. These properties of proteins are due to their *amphoteric* quality, that is to say, their capacity to form salts in the presence of either acids or bases.

The amphoteric properties of proteins may be stated thus: in the presence of acids, proteins form salts in which the protein ion is positive:

$$\text{Albumin} + HCl = AlbCl \rightarrow Alb^+ + Cl^-$$

In the presence of bases proteins form salts in which the protein ion is negative:

$$\text{Albumin} + NaOH = NaAlb \rightarrow Na + Alb^-$$

All major properties of proteins are determined by their acid-alkaline condition, usually expressed as the *p*H of the solution.

During studies on the cataphoretic migration of protein particles (page 18) W. B. Hardy found that proteins carry a positive charge when dissolved in acid and a negative charge when dissolved in alkali. A change of charge could be brought about simply by adding dilute acid or alkali. Precipitation took place at the *isoelectric point*, i.e., the point where the sign changed and no charge was carried. Some proteins, however, do not flocculate at the isoelectric point; there is then the wider definition that the isoelectric point is the point of no cataphoretic migration or of least density of electric charge. The isoelectric point is usually expressed in terms of *p*H, because the degree of acidity usually determines it in proteins. If, however, zero potential can be reached by some means other than through a change in acidity, for example, by adding salts of high valence, then the isoelectric point is no longer a *p*H value, and it is erroneous to define it as such. Inability to form salts with either acids or bases is characteristic of a substance at its isoelectric point. This point may be given, then, in terms of *p*H when acidity is responsible; but it may also be expressed in terms of potential, stability, or turbidity.

The isoelectric point of gelatine, in terms of acidity of the solution, is *p*H 4.7; and of globulin, *p*H 4.2. The isoelectric points of most proteins are between *p*H 3.5 and 5.5. As the *p*H of protoplasm is nearly always above 5.5—between 5.5 and 7.4—the proteins in cells must, therefore, be negatively charged, and behave as anions. This explains why all living cells are negatively charged when in their natural solutions, for body fluids are on the alkaline side of the isoelectric points of proteins. The *p*H of plant-cell sap is usually about 6, of blood 7.38, and of the ocean on the average 8.2.

Evidence that the proteins are fundamental in life and to a great extent determine the character of an organism is to be had from the serological studies of Mez on plant relationship. When a vegetable or animal protein, or *antigen*, is injected into an animal of a different species, a precipitating *antibody* is formed. The intensity of the precipitation reaction between these antibodies and plant and animal proteins is an indication of the nearness of their interrelationships. Using such methods, Mez has constructed a plant family tree which corroborates almost fully the older classification based on taxonomic (morphological) characters. Where differences in results by the two methods exist, the correct relationship is just as likely to be shown by the newer serological reactions as by the older anatomical evidence. It is of great significance to the field of evolution and phylogenetic relationship that a purely chemical basis of classification should so well support a purely anatomical one. Relationships between plants established by serological methods hold well for families but not so well for genera and not at all for species. This is due simply to a lack of delicacy in the technique; species differences in proteins must, of course, also exist (see also page 256).

FATS

When tissues are subjected to solvents such as ether, chloroform, and acetone, substances which are greasy to the touch are extracted. These are *fats*, esters of the fatty acids combined with glycerol or other higher alcohols.

Fats are ordinarily regarded as sources of reserve food without other use of primary importance, but their role in life is by no means simply stored food; the fats hold a prominent position in physiology.

Fatty substances include fats, oils, waxes, lipins, lipides, lipoids, phosphatides, sterols, hydrocarbons, and essential oils. The hydrolysis of fats is *saponification*; it results in the formation of a soap. Fats, fatty acids, and soaps are widely distributed in plants. Soaps may serve as stabilizers of the protoplasmic emulsion.

There is probably little if any movement of fats in the plant. They are synthesized by specific enzymes in the cells where used or stored, and apparently are formed from carbohydrates, as indicated by the conversion of large quantities of starch in young seeds to oil in ripe seeds. The kernel of the almond contains the following percentages of material when young and when old:

	Oils	Sugars	Starches
June	2	13	22
October	46	3	5

Just as fats arose from the carbohydrates, so does it seem likely that they are again reduced to carbohydrates for transport and utilization.

Among the ether-soluble constituents of tissues are substances which have some but not all the properties of fats. This artificial group is known as the *lipoids*, and may be subdivided into the more natural groups, *phosphatides* and *sterols*. *Lecithin* is a complex nitrogen and phosphorus-containing, fat-like substance, generally classed as a lipoid, phosphatide, or phospholipide, and is widely distributed in plants.

Physiologists have long attributed to the fats and fat-like substances an important role in cellular permeability (see page 122). Lecithin has been given a particularly prominent place as a constituent of the protoplasmic membrane. Of special interest, too, are the surface processes or papillae put out by cells; these resemble the *myelin* forms produced when lecithin comes into contact with alkalies or acids.

Sterols are solid alcohols with a hydroxyl group. *Cholesterol* ($C_{27}H_{45}OH$) is the chief sterol of animals. As cholesterol does not occur in plants, and as the several plant sterols are not common in animals, there appears to be a distinction between the plant and animal kingdoms in regard to their sterol content. If this is true, then the position of groups which are near the border line of the two kingdoms may be established on the basis of their cholesterol content. Chemical analysis of the myxomycete, *Physarum polycephalum,* has yielded cholesterol, thus loosening the hold of botanists on slime molds!

Ergosterol is essentially a plant sterol, but occurs in certain animals as well. It was discovered in ergot of rye, *Claviceps purpurea,* by the French apothecary, Tanret, in 1888. Ergosterol when irradiated with ultraviolet light is converted into vitamin D, and has, therefore, assumed considerable economic importance. Three of the five substantial vitamins are now known to be associated with fats—the growth vitamin A, the antirachitic vitamin D, and the reproductive or fertility vitamin, E.

As activators and organizers of bodily functions, the fats are encroaching upon the proteins.

VITAMINS

Vitamins may be regarded as higher foods; but, as they are needed in such minute quantities, their function may be more that of an activator, or catalyst, than of food. Foods serve their use as stored energy, but this is not true of vitamins. One need only compare the quantity of sugars, starches, proteins, and fats taken in by an animal, with the trace of vitamins necessary to keep it in good health, in order to realize that whereas vitamins are generally considered to be foods, in the sense that they are nutritive, they are in a class by themselves. There is today no more active and important branch of animal nutrition than the study of vitamins.

A vitamin is any substance that is indispensable for normal growth and good health in respect to some typical function, such as the prevention of a specific disease. Six of them, lettered A to G, are now generally recognized. Plants are the chief source of vitamins. Vitamins, as definite substances, were unknown before the present century. Not until 1897 was the first successful experiment on vitamins made, and the significance of that experiment was not fully understood until as recently as 1910.

Although plants are the chief source of vitamins, knowledge of their physiology has come solely through studies on animals.

The six recognized vitamins, their chief sources, and the diseases they prevent are:

Vitamin	Sources	Diseases and Nutritional Disturbances Prevented
A	cod-liver oil, milk, egg yolk, carrot	night blindness, lowered resistance, and retardation of growth
B	yeast, embryos of grain	injury to nerve tissue, loss of appetite
C	paprika, lemon	scurvy
D	cod-liver oil, egg yolk, butter	rickets
E	wheat embryo oil	sterility
F	See note	
G(B_2)	yeast	beriberi, pellagra

Note: Once the precise chemical constitution of a vitamin is known, the tendency is to drop the vitamin designation and call the substance by its chemical name. This is what has happened to vitamin F; it is linoleic acid. It stimulates growth. It is the only fatty acid which the animal body needs and cannot synthesize.

The disturbances caused by the deficiency of a vitamin are not as specific as originally thought. The lack of any one vitamin is certain to cause not one but a number of disorders; thus, normal growth depends upon the presence of thirty or more indispensable nutrient substances. Nor will any vitamin, for example, the antisterility vitamin E, accomplish more than keep man an average human being.

Among the vitamins, one, vitamin G, has been synthesized; it is identical to *ascorbic acid.* The structural formula of three others, A, B, and D, [omitting F] are reasonably well known. Vitamins C and B are now commercially available in crystalline form.

The vitamins, or their precursors, are primarily plant products. Vitamin A is produced through conversion of the plant pigment carotene by the liver. Ergosterol—first found in ergot of rye and now commercially produced from yeast—is a provitamin D of which there are several.

Plant physiologists may be called upon to supply a source of certain vitamins for man and lower animals, but their main interest is the possible need of the plant for the vitamins it synthesizes.

How far vitamins are significant for plant life cannot be said with certainty. The difficulty lies in experimentation. Animals must be fed organic food, the vitamin content of which can be controlled, but green plants require only inorganic nutrition from which they manufacture their own food, including vitamins. The need of vitamins can only be surmised so far as green plants are concerned, but in the saprophytic plants, or certain non-green parts of chlorophyll-containing plants, the need of vitamins may be experimentally ascertained. If the root or other colorless part of a green plant requires vitamins, it is very likely that all parts of the plant also require the same vitamins.

The need of vitamins for plant growth is proved for roots in culture as far as vitamin B is concerned. Bonner, Robbins, and P. R. White have each shown that this vitamin is essential for the growth of excised root tips (page 242). Vitamin B is, therefore, not only an animal vitamin and a growth substance for fungi and bacteria, but it is also required for the proper growth of higher plants.

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CHAPTER XVII

CELLULOSE AND THE CELL WALL

The plant framework is formed by the walls of cells, and these consist largely of cellulose. The manner in which the cell wall is laid down, its composition—for it is not pure cellulose—its structure, and other properties, are of botanical interest and of economic importance. The texture, tensile strength, wearing qualities, and other properties of cellulose objects now in common use are in part predetermined by the structure and composition of the cell wall.

Cellulose is not a single chemical entity, for there are numerous celluloses. The nearest approach to a pure cellulose in nature is cotton fiber, which is 90 per cent "alpha" cellulose. Higher celluloses are synthesized by the plant from lower carbohydrates, and in the process certain intermediate products which resemble cellulose are formed. Such is hemicellulose; it is a form of cellulose which may possibly serve as food to both plants and animals. Nutrition is a doubtful, and in any case a minor, use of cellulose compared with its role as the skeletal material of plants.

Something of the gross morphology of the plant cell wall was known to the earlier botanists. Naegeli in 1864 described the lamellated structure of the cell wall (*A.*, Fig. 47). He also noted the striated structure of the lamellae as seen in surface view, the crossing of the striae of overlapping layers at an angle of 45° (*C.*, Fig. 47), and the spiral twisting of the cellulose threads (*B.*, Fig. 47).

All these observations have been substantiated by later work involving different methods of research. The lamellated structure of the cell wall as seen in cross section has been investigated by I. W. Bailey using ordinary methods of staining and, in particular, polarized light. The latter has revealed striking pictures. The cell wall consists of three, sometimes four, main parts (Fig. 48). There is, first, the middle lamella, about which there has been much controversy; then, the thin primary wall; next, the heavy secondary wall which forms the major part of the cellulose layer; and occasionally there is a tertiary wall. The secondary wall is itself lamellated. The lamellae are vividly brought out by polarized light, which shows them to be of optically different material, isotropic layers alternating with anisotropic ones (Fig. 49).

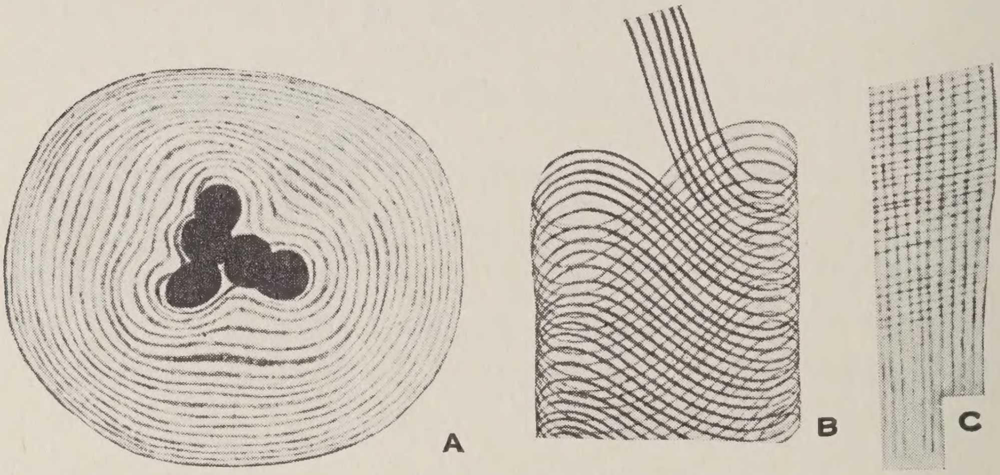

FIG. 47. Cell wall structure: *A,* concentric layering; *B,* fibrils; *C,* overlapping cross striations. (From C. Naegeli.)

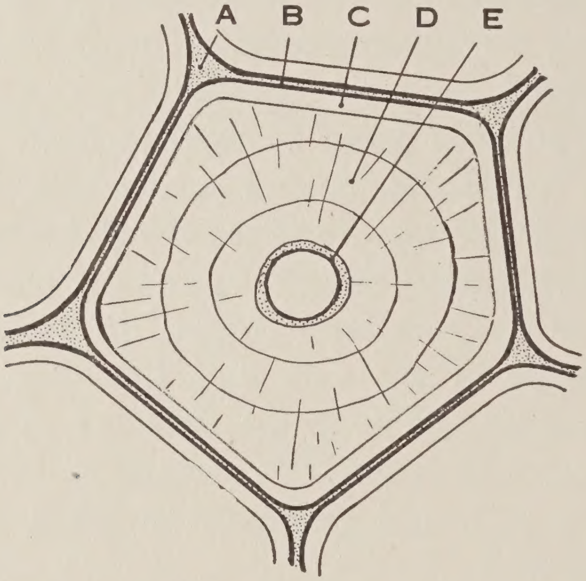

FIG. 48. The layered wall of a sclerenchyma cell: *A,* intercellular material; *B,* the primary wall; *C,* the secondary wall; *D,* successive thickenings of the secondary wall; *E,* tertiary wall. (After I. W. Bailey.)

The lamellated structure of the cell wall is made visible by other methods, such as maceration and swelling. The concentric layers are often very numerous. Balls has shown that there are twenty-two layers in cotton fiber, which indicate the age of the fiber, for the fiber requires twenty-two days to reach maturity. The layers are, therefore, laid down daily. Anderson has shown that continuous illumination eliminates the diurnal layering (Fig. 92).

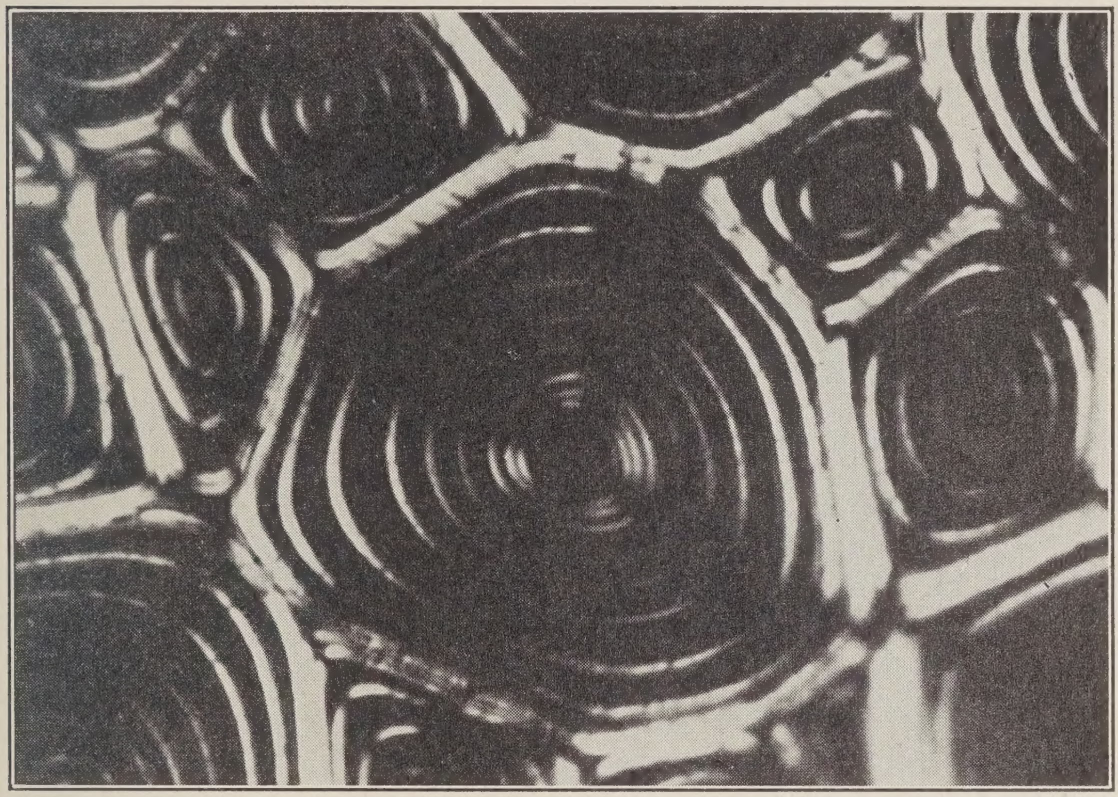

FIG. 49. Alternate birefringence in fibers of *Pandanus odoratissimus*; isotropic layers of the cell wall are brilliantly illuminated; anisotropic layers are black when seen with polarized light through crossed nicol prisms. (From I. W. Bailey.)

The lamellae are concentric layers fitting together like cylinders, one within the other. These cylinders are built of striae or fibers, which lie side by side, forming a sheet of cellulose; and the fibers are built of shorter units or rods joined end to end, sometimes overlapping one another. The striated structure of the lamellae is to be seen in light-field illumination (Fig. 47C), but is more strikingly revealed by dark-field illumination (page 17, Fig. 6). Viewed with dark-field, the cell wall is shown to be a striated structure of almost perfect symmetry (Fig. 50 left). Strains and stresses disturb the uniform parallelism of the striae at certain points. The striae or fibers are seen to consist of particles, or short rods, about 2 μ in length. The X-ray work of Preston and Astbury supports the reality of the striations seen on the walls of cells when illuminated by indirect light. These, therefore, are not artifacts but correspond closely to the structure of the wall.

The same symmetrical orientation of striae composed of short units is to be seen in coal when viewed against dark-field (Fig. 50 right). Thus is the structure, established when the cell wall was originally laid down by the protoplasm, maintained in fossil cellulose.

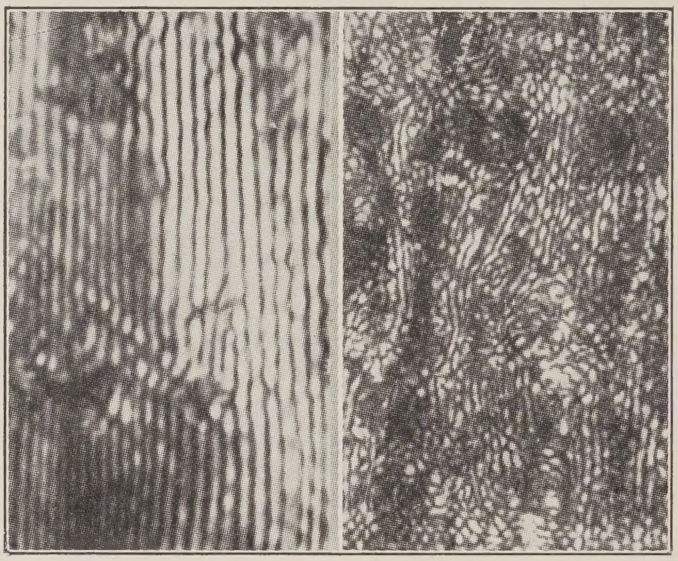

FIG. 50. Dark-field (Spierer lens) photographs of cell wall, left, and coal, right. (Coal photograph from R. Thiessen.)

That the striae seen in dark-field represent actual fibers is supported by all that is known of the structure of wood. G. Ritter, by maceration and dissection, and C. Hock, by the microdissection of paper-pulp fibers, have shown that the microscopic pictures of natural cellulose are substantiated by anatomical analysis (Fig. 51). When a fiber is entered by microneedles and these then separated, the fiber is split lengthwise, indicating that it is not a homogeneous mass but consists of linear strands. If a microneedle is made to enter the fiber very close to its edge and then moved away, a single fibril may be lifted off (Fig. 51). Careful micromanipulation reveals that the fibrils, which compose the larger strand, are themselves bundles of fine threads, so delicate as to defy individual handling. These secondary fibrils approach the limit of microscopic visibility.

The spiral twisting of cellulose fibrils (*B.*, Fig. 47) has been demonstrated by many workers. Scarth has pictured the spirality of fibrils in a bast fiber (Fig. 52).

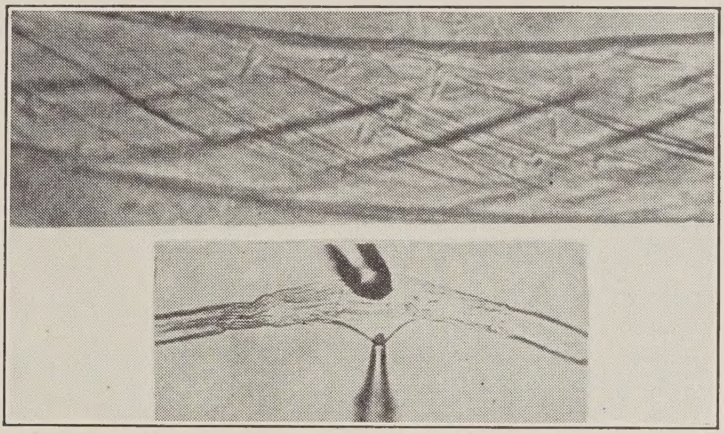

FIG. 51. Spiral fibrals in a paper-pulp fiber (top). Microdissection of a single fibril from a paper-pulp fiber (bottom).

Turning now from the gross morphology of the cell wall to the submicroscopic and molecular structure, one has further evidence of a fibrous structure in cellulose with complete agreement and mutual support of the microscopic, ultramicroscopic, and theoretical results. The botanist Naegeli postulated a *micellar* structure of gels. *Micellae* are fascicles or bundles of molecules. As molecular aggregates or micellae are below microscopic visibility but above molecules in size, the micellar hypothesis is therefore a colloidal one. The cellulose micelle is a bundle of linear molecules, crystalline in nature; for this reason it has received the name of *crystallite* (Figs. 53, 54). The precise orientation of the micelles in cellulose is not known; it apparently differs from one form of this substance to another. One postulate states that the micellae are arranged like bricks in a wall (Fig. 53). Dark-field pictures of living plant cell walls support this (Fig. 50 left).

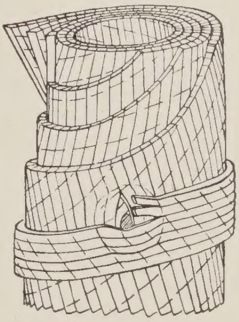

FIG. 52. Arrangement of lamellae, striae, and spiral fibrils in a cell wall. (From G. Scarth.)

The micellar theory is widely accepted as an interpretation of the structure of gels and jellies (page 13). Whereas gels or coagula of the silica type are most certainly micellar or finely granular in structure, it may be that jellies, such as gelatine, are not strictly colloidal but molecular, in that their structural unit is a molecule of large size. Which situation occurs in cellulose cannot be said; rod-shaped colloidal micellae and molecular threads have both been postulated. Possibly the state of aggregation of the chain molecules differs under different conditions so that either a micellar or a molecular structure may exist.

The term micelle is usually retained for ultramicroscopic particles, the evidence of their existence being indirect; but if the term is extended to include minute molecular aggregates of microscopic dimensions, then there is direct visual evidence of their existence, namely, the 2 μ particles seen in the cellulose walls of plant cells (Fig. 50 left). Micelles must be as long as the molecules which compose them, for one cannot make a bundle of sticks shorter than the sticks. It has been maintained that cellulose molecules may attain a length of 1.7 μ; if true, micelles then become microscopically visible. However, in order not to confuse microscopically visible units with the smaller ones which the cellulose chemist terms micelle, the larger particles may be referred to as *supermicelles*.

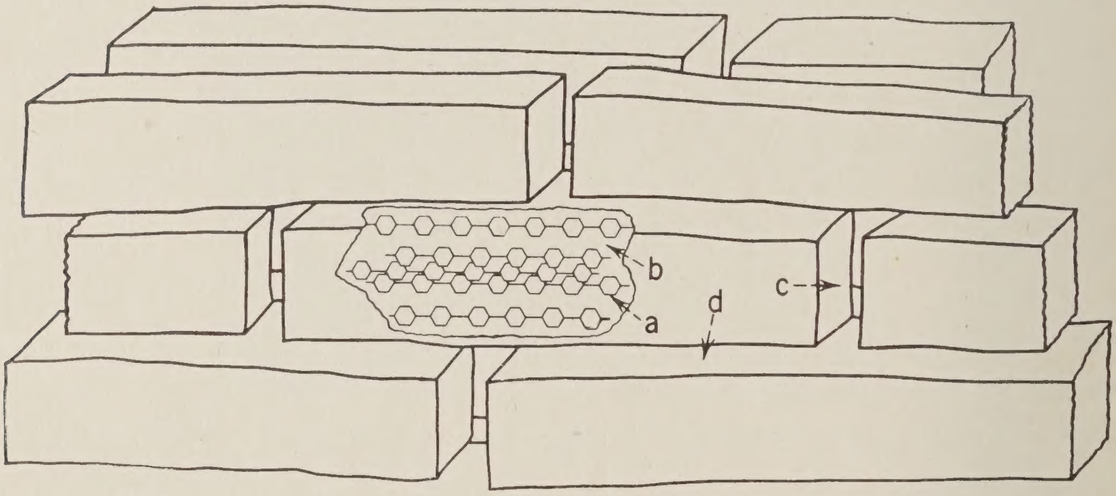

FIG. 53. Orientation of micellae in cellulose: a, b, c, and d indicate types of valence.

Further evidence of a micellar structure in natural cellulose is to be had from the work of Fry-Wyssling, who, with the aid of polarized light, produced by crossed Nicol prisms, established the general direction of the three planes of symmetry and concluded that in cell walls there are submicroscopic or colloidal rod-shaped particles, which he identified with the Naegeli micelles.

Sponsler and later workers have shown, with the aid of X-ray analysis, that the cellulose molecule is a chain of anhydrous glucose rings of the familiar formula, $C_6H_{10}O_5$. These rings are united by an oxygen bridge, each successive ring being rotated through 180°, that is to say, each is the mirror-image of its two neighbors (Figs. 54, 55). The cellulose chain thus formed is not of fixed length—the law of the constancy of molecular size and weight is, therefore, not adhered to. A minimum number of forty glucose residues is attributed to the molecule, which means a total length of 200 A. U. (0.02 μ). Conservative maximum lengths of the cellulose molecule place it at 2000 A. U. with a

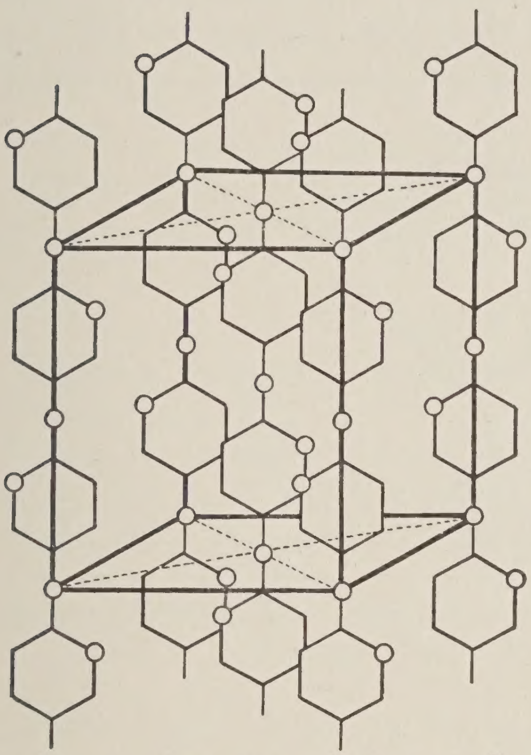

FIG. 54. Part of a cellulose micelle or crystallite. (After Meyer and Mark.)

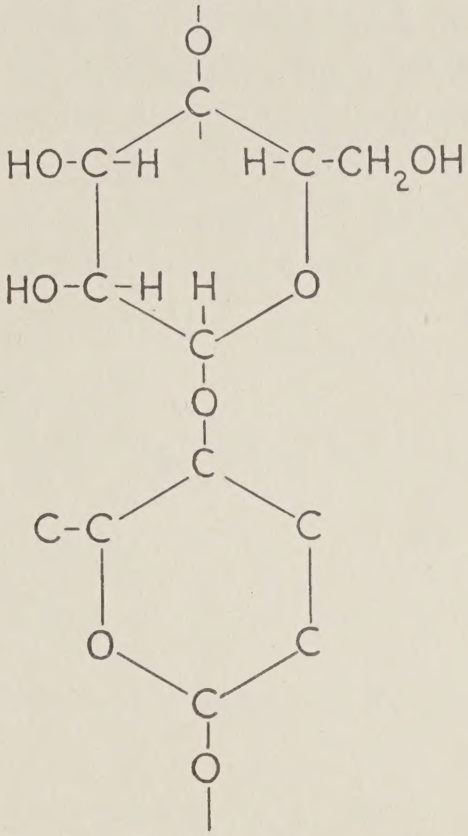

FIG. 55. Two rings in the chain of the cellulose molecule.

weight of 200,000. The startling length of 17,000 A. U. (1.7 μ), with a possible maximum molecular weight of 500,000, is said to be attained in some cases. The cross section of the molecule remains of atomic dimensions, 5 A. U.; it, therefore, is not visible no matter what the length may be. The chain molecule behaves as a comparatively stiff thread.

It is these long fibrous molecules of cellulose which are presumed to be aggregated into fascicles or micellae. That they may not always be so has been suggested. There are other possible arrangements of the molecules which better explain such properties as tensile strength and elasticity. Perfect parallelism with overlapping of the individual molecular fibers is possibly present in native ramie, which is of relatively high tensile strength (*A.*, Fig. 56). Random or "brush-heap" distribution probably occurs in cellophane of lower tensile strength (*B.*, Fig. 56). The cellulose of flax displays an excellent orientation of molecules or micelles parallel to the fiber axis, which accounts for its very high tensile strength.

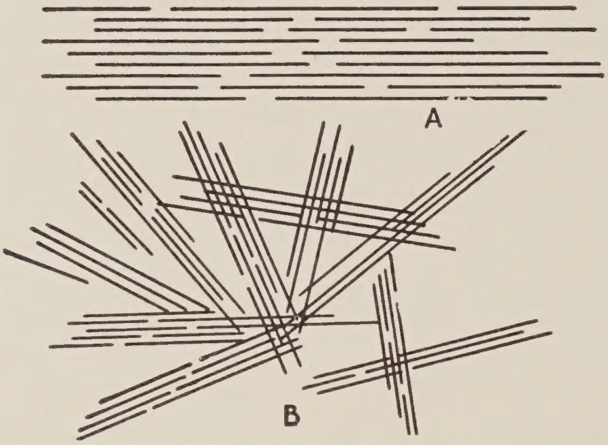

FIG. 56. Arrangements of micellae or of chain molecules in cellulose: *A,* orderly parallel overlapping; *B,* "brush heap."

There are many polymeric materials which are constructed on the same principle as cellulose in that their molecules are characterized by a chain of recurring units. Those of an organic nature, which have been subjected to X-ray study and found to be crystalline in nature with linear units in orderly arrangement, include starch, gelatine, chitin, rubber, silk, hair, keratin, sinew, muscle, nerve, and brain. It is but a step from these to protoplasm; indeed, muscle, nerve, and brain actually are protoplasm. Thus does the structure of cellulose as determined by X-ray analysis throw light on the structure of living matter.

The most encouraging feature of the work done on cellulose and cell-wall structure is the complete agreement, mutual support, and interlocking of the results obtained by various methods. Whether it is the indirect method of the X-ray chemist or the direct observations of the microdissectionist, all evidence points to a fibrous structure in natural cellulose. The fibers range from long molecular threads, a few Ångströms in diameter, to microscopically visible fibers from which larger strands such as twine and rope are manufactured.

The length of the fibers—molecular, colloidal, or microscopic—their stratification and their twisting, are all problems in plant structural "engineering."

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CHAPTER XVIII

PLANT PRODUCTS

ENZYMES

Enzymes, from the Greek *en zymos*, meaning "in yeast," were formerly known as *ferments* and were regarded as of two kinds, those involving entire microorganisms, such as yeast and bacteria, and those secreted by higher animals, as are saliva, gastric juice, and pancreatic secretions. The latter were described as "unorganized," non-living ferments "of a chemical nature."

Although some investigators early maintained that a distinction between living and non-living ferments was unwarranted, this contention was not proved until 1897, when *zymase* was isolated from yeast and was found, even after all trace of living matter had been removed, to bring about the same fermentative processes which heretofore had been held possible only in the presence of living cells. An enzyme, therefore, may be defined as a ferment produced by plant or animal cells, its properties being in no way dependent upon the life of the cell. Enzymes are thus all of organic nature and all products of living matter. They function by *catalysis*. *Catalysts* are substances which bring about or accelerate chemical reactions but appear unchanged among the final products. It is not possible to distinguish sharply between inorganic catalysts and organic catalysts, or enzymes, other than in regard to their origin.

One of the remarkable qualities of enzymes is their *specificity*, which limits them to a specific substance or substrate. For every synthesis and decomposition that occurs in living processes, a specific enzyme presumably exists. The two major groups of enzymes are the *anabolic* and *catabolic* ones, i.e., those which build up and those which break down. How enzymes function is not known.

An important enzymic reaction in life, that which reduces sugars, may be indicated by the following reaction—this is, however, a very simple representation of what is probably a very complex process:

$$\underset{\text{sucrose}}{C_{12}H_{22}O_{11}} + \underset{\text{water}}{H_2O} + \underset{\text{sucrase}}{\text{enzyme}} \rightarrow \underset{\text{glucose}}{C_6H_{12}O_6} + \underset{\text{levulose}}{C_6H_{12}O_6} + \underset{\text{sucrase}}{\text{enzyme}}$$

A few of the well-known enzymes, the substrates they attack, and the end products formed are given in the following list:

ENZYME	SUBSTRATE	END PRODUCT
sucrase	sucrose	levulose and glucose
maltase	maltose	glucose
diastase	starch	maltose
cellulase	cellulose	cellobiose
lipase	fats (lipins)	glycerol and fatty acid
pepsin	protein	proteoses and peptones
trypsin	protein	polypeptides and dipeptides
chlorophyllase	chlorophyll *a*	chlorophyllide and phytol
zymase	hexose	ethanol and carbon dioxide
catalase	hydrogen peroxide	water + oxygen

Cellulase is an important enzyme for fungi; it digests the cellulose walls of plants. The ability of fungi to enter the cells of plants is due to their power to secrete cellulase. No higher animal possesses any digestive ferment capable of attacking cellulose. The wood-eating capacity of animals, such as termites, is due to the presence of bacteria or protozoa in their digestive tracts. Cellulase or some other cellulose-attacking enzyme is secreted by these bacteria and protozoa.

The fact that fungi, and possibly other plants, are able to convert cellulose into sugars suggests that celluloses may be used by plants as reserve food (page 178).

The specificity of enzymes is shown in maltase, which hydrolyzes maltose. Maltose is a disaccharide like sucrose—the two are isomers, identical in chemical composition, but differing in atomic arrangement. Their structural differences are sufficient to require specific enzymes; sucrase will not digest maltose nor will maltase digest sucrose.

Several enzymes, e.g., trypsin, pepsin, and urease, have been obtained in the crystalline state. Until the crystallization of an enzyme had been accomplished it was impossible to say if any were homogenous and to what group, protein, carbohydrate, etc., they belonged.

Willstätter, a pioneer worker in enzymes, suggested that enzymes consist of two components, a *colloidal carrier* and an *active* group. The carrier was thought to be a protein, its function being unknown other than to serve as a substratum to which the active principle is attached. Hemoglobin illustrates the situation; globin is the protein carrier and hemin the active component.

The foregoing hypothesis still holds, but in quite a different form from that originally intended by Willstätter. The carrier is now known to be specific, which means that not any carrier will do. Furthermore, neither the carrier nor the active or so-called *prosthetic* group will function as a catalyst when alone. Each requires its partner, and there is no reason to regard the one as any more important than the other. Viewed in this light, the protein component becomes as significant as the prosthetic or non-protein component. It is the whole which is the enzyme.

Enzymatic activity may be inhibited by *paralyzers* or accelerated by *activators.*

Although the mechanism of enzymic action is not known, hypotheses are not lacking. That the enzyme establishes an environment suitable to the reaction is the simplest suggestion. The enzyme may present a surface upon which the reaction can take place. It is for this reason that spongy or colloidal platinum is such an excellent activator in contrast to metallic platinum. Although the definition of a catalyst requires that it shall not occur as an integral part of the end product or be used up, yet it is possible that the enzyme enters into the reaction but withdraws before the end. The theory that there is an actual combination between the enzyme and its substrate is widely accepted, but it is not known whether the union is a molecular one or not.

Enzymes possess properties closely akin to living matter; thus they are "killed," i.e., inactivated, by heat. It has been suggested that protoplasm is an aggregation of enzymes. That enzymes are fundamental parts of protoplasm is indicated in recent work by Bergmann and Niemann, who point out that, as the building of protein molecules proceeds according to a well-defined plan, there is no other choice than to assume that the synthesis is controlled by specific chemical organizers, or enzymes, which are thus fundamental to life.

PIGMENTS

In an old textbook on physiology, the following statement may be found: "A vast number of waste products occur in plants such as organic acids, alkaloids, aromatic substances, and coloring matter." This remark was permissible half a century ago when little was known of the coloring matter in plants, but today pigments are no longer regarded as waste products; they have risen from this position to one on a level with the most significant of vital substances.

Among plant pigments, chlorophyll is the most outstanding one. Its importance has been emphasized (page 140).

A second plant pigment, *carotin* ($C_{40}H_{56}$), has assumed a position of physiological importance through its nutritional value; it is associated, if not identical, with vitamin A. Carotin is one of the so-called *carotinoids*. These include most of the yellow pigments of plants, two of which, carotin and xanthophyll ($C_{40}H_{56}O_2$), invariably occur with chlorophyll. The "lipochromes" of animal tissues are probably identical with carotinoids; both are produced by plants. The yellow matter of butterfat and of the adipose tissue of cattle is carotin; the pigment of the yolk of eggs is xanthophyll. (Carotin is often spelled carotene in chemical literature.)

The *flavones*, which are yellow pigments of wide distribution in plants, have long been utilized as vegetable dyes. The important dye *hematoxylin*, from the tropical American tree, *Haematoxylon,* is a flavone. The flavones are structurally related to the anthocyanins. The *flavins* are quite distinct chemically from the flavones, though similar in name. They occur in very small quantities and are of considerable physiological significance through the role which they play in respiration (page 165).

Yellow pigments in general are abundant in plants and many may serve important functions as yet unknown, as for example, the yellow pigment of slime molds (page 97).

Anthocyanin is another pigment of biological value. It is the basis of red and blue colors in flowers and fruits. The term "anthocyanin" was first used for the blue pigment of flowers. Later the red and purple pigments were found to be of the same chemical constitution, existing in a different form. Anthocyanin is now generally used to indicate the red, magenta, and blue glucoside pigments, and "anthocyanidin" the non-glucoside form.

Although the part played by most pigments is not known, the important position held by chlorophyll in plant life, by hemoglobin in animal life, and by the yellow respiratory pigment in the life of possibly all cells raises pigments out of the category of waste products into a place of prime importance in life.

ALKALOIDS

Alkaloids are not abundant in plants but include some very familiar compounds, quinine from *Cinchona*, cocaine from *Erythroxylon coca*, morphine from *Papaver*, strychnine from *Strychnos nux-vomica* being but a few.

The natives of the Indo-Malay countries are addicted to the habit of betel nut chewing, and the Indians of the South American Andes chew coca leaves. The nuts and the leaves yield an alkaloid; in the case of coca it is cocain. The natives add a small quantity of lime, often simply powdered sea shells, to the leaves, the purpose of which is usually a puzzle to the casual observer. The alkaloid in the nut and leaf will not go into solution except in an alkaline medium. The lime supplies the alkaline medium.

Whether or not alkaloids are to be regarded as useful to the plant will depend on whether or not a use for them is known, and as yet this is not the case. The cinchona tree is said to produce no quinine when grown in hothouses, the implication being that its alkaloid is not necessary for good growth. Where functions are unknown, resort is often made to such a function as activator and regulator of reactions; this has been done in the case of alkaloids. The toxicity of alkaloids may be of value to the plant as protection against attack by insects and higher animals.

It is not necessary to seek a function of plant constituents in a positive sense, for waste products play a role no less important. Alkaloids may hold a place in the physiology of plants comparable to that of urea in animals.

GLUCOSIDES

As their name indicates, the glucosides are compounds containing glucose; rarely, other monosaccharides take its place. The sugar is bound to a non-sugar residue which is often an aromatic substance. Some thirty or more glucosides are known; among them are amygdalin from bitter almonds, quercitron from the bark of oak (here the sugar component is not glucose but rhamnose), and digitonin from foxglove, which can be broken down into its component parts, thus:

$$\underset{\text{digitonin}}{C_{54}H_{92}O_{28}} + 2H_2O \rightarrow \underset{\text{glucose}}{2C_6H_{12}O_6} + \underset{\text{galactose}}{2C_6H_{12}O_6} + \underset{\text{digitogenin}}{C_{30}H_{49}O_6}$$

Saponin (from Panama wood) is one of a group, the saponins, which are soap-like substances that are readily dispersed in water, forming colloidal suspensions. They have a variety of uses (cleansers of cloth, emulsifiers, etc.). Digitalis (or its chief constituent digitonin) is a saponin. The saponins are all poisons, some being highly toxic.

The glucosides occur in cell sap and appear to be waste products. One function of the glucosides which has been suggested is to hold substances of great physiological activity inert until they are needed. With this exception, the same conclusion is reached for the glucosides as for the alkaloids.

TANNINS

Tannins were formerly classed with the glucosides because of the sugar and non-sugar groups which some of them possess, but they are now usually considered in a class by themselves, since certain of them, such as the hemlock tannins, lack the sugar group.

Emil Fischer accomplished the synthesis of a tannin, or tannin-like substance ($C_{220}H_{142}O_{58}N_4I_2$), with a molecular weight of 4021, which, regardless of the nature of the substance, is of interest as it is the highest molecular weight ever attained for a synthetic substance. The polypeptide synthesized by Fischer had a weight of 1268. Far simpler are the earlier formulae given for tannin (e.g., $C_{34}H_{28}O_{22}$).

Tannins are widely distributed in plants, chiefly in vacuoles and in cell walls, where they have been absorbed as the cell grows old and loses its protoplasmic contents. Tannins are particularly abundant in leaves, fruits, and plant galls. Tannins are very astringent, as any one who has eaten an unripe persimmon can testify. Their use to plants, like that of their close relatives, the glucosides, is wholly unknown. Again the suggestion has been made that they are protective, being distasteful to animals. As animals, by eating fruits, are important agents in seed dispersal, only the unripe fruit need be distasteful to them. On ripening, the tannins are usually oxidized or otherwise destroyed.

ETHEREAL OILS

The odor of flowers, leaves, and stems, whether the sweet perfume of violets, the pungent odor of spices, or the obnoxious odor of the skunk cabbage, is due to *ethereal* or *essential oils*. All are characterized by their volatile nature, which is evident from the distance at which an orange can be smelled when being peeled. The scent of the honey locust and the tropical wild ginger can be detected many yards away. The extracts from rose petals, orange blossoms, jasmine, and many other flowers used in the making of perfumes are all ethereal oils.

The ethereal oils containing oxygen are aldehydes, ketones, phenols, and acids of wide distribution and great variety. They include menthol, camphor, coumarin, to which the odor of new-mown hay is due, and the active principles of the oils of cinnamon, wintergreen, cloves, thyme, and eucalyptus.

The ethereal oils not containing oxygen are hydrocarbons called *terpenes*. They give the characteristic odors to turpentine from pine trees, oil of lemon, and oil of peppermint.

Possible functions of ethereal oils are again only vague suggestions, of which the attraction of insects and other animals aiding in pollination is one.

RESINS

Coniferous trees commonly secrete resins of various kinds in combination with gums, as well as ethereal oils. Canada balsam is a mixture of true resin, ethereal oils, and aromatic acids. Amber is a fossil resin.

The function attributed to resins is again one of protection; they serve in the preservation of old wood. Here the evidence is certainly more convincing, for pitch pine is more resistant to the attack of fungi and insects than wood not impregnated with pitch. Furthermore, the resins are most abundantly secreted at the site of a wound, where they undoubtedly protect from insects and bacteria.

LATEX

A considerable number of plants secrete a milky fluid known as *latex*, which in time, on exposure to the air or on the addition of an acid, coagulates. Certain latices coagulate into a very elastic substance known as crepe or crude rubber. Rubber latex is secreted by a variety of plants, including the fig, *Ficus elastica,* which was the original source of "India rubber," the milkweed, *Asclepias,* the spurges, *Euphorbia,* and the South American tree, *Hevea brasiliensis,* the present source of most commercial rubber. The hydrocarbon in natural rubber is *isoprene* (C_5H_8).

Similar in constitution to rubber is *gutta percha,* or *caoutchouc* as it is known in the crude form; it is the coagulated latex of several tree members of the Sapotaceae.

Latex is secreted by a number of other plants, such as the bloodroot, *Sanguinaria,* the red fluid in which is a pigmented latex, and several composites, the dandelion *Taraxacum* being a familiar one.

The survival of plants containing latex may be interpreted as due to the stickiness and acridness of their latices, which would protect against herbivorous animals. The latex of the upas tree, *Antiaris toxicaria,* is highly toxic and is used by the natives of Java for poisoning their arrows. Latices also contain numerous complex organic compounds and may thus serve as food reservoirs.

NECTAR

Nectar is secreted by nectaries or groups of glandular cells located close to the end of a fibrovascular bundle, so situated in the base of a flower that in order to reach it an insect or humming bird must brush past the stamens and thus become coated with pollen. This suggests that the function of nectaries is to attract insects and insure cross-pollination. The function might better be stated in this way: the plant, having a waste product which is sweet to insects, is, as a result, cross-pollinated.

There are other plant products of interest and importance, such as the secretion in the tips of the stinging hairs of the nettle (Fig. 17) which protects the plant from animals, and the secretions of insectivorous plants (see page 279) which function as digestive ferments; but our list is already a long one.

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CHAPTER XIX

HORMONES AND GROWTH REGULATORS

HORMONES

Regulators of plant growth were postulated by the botanist Sachs; today these substances are known as *hormones*. The name hormone was originally applied to the secretions of the endocrine glands, but all cellular secretions, in so far as they function as regulatory substances, are now classed as hormones. They exert an influence on growth and development by controlling specific chemical reactions, possibly functioning as catalysts. In plants, the study of hormones has dealt primarily with growth, wound healing, and regeneration; thus, Haberlandt postulated specific wound-healing hormones in plants. Previously, Darwin had speculated on the cause of phototropic and geotropic response in plants and suggested the presence of a growth-promoting substance or influence. He was the first to show that in many instances the action of an external agent, such as light or gravity, may affect one part of an organ, whereas the response, phototropic, geotropic, or other, occurs in a part which is often separated from the first by a distance of several centimeters. Darwin concluded that a stimulus is transmitted through the tissues of the plant. Today the "stimulus" postulated by Darwin is known to be a hormone; he had stated the same idea in other words. He found that if the tip of the coleoptile of a seedling is illuminated on one side, curvature takes place below the tip; he concluded that "these results seem to imply the presence of some matter in the upper part which is acted on by light, and which transmits its effect to the lower part."

It is now generally recognized that phototropic and geotropic curvatures and similar movements are due to the unequal growth of opposite sides of a plant or plant part. The cause of the unequal growth is attributed to a hormone, formed in the plant and accumulating toward one side of it. This may be a consequence of the polarization of the tissues induced by the one-sided illumination.

In 1910 Boysen-Jensen inserted a mica plate part way into a coleoptile and then illuminated the seedling on one side. He found that curvature toward the light took place. When, however, the back or shaded half of the stem was severed, no bending resulted. He concluded that a growth-stimulating substance migrates down the opposite side of the stem from that illuminated, promoting growth curvature toward the light. Movement of the hormone took place even after all living connection between tip and base had been destroyed. The tip of the coleoptile was then removed, a drop of gelatine placed upon the cut surface, and the tip replaced in its original position. Positive phototropic curvature took place below the replaced tip just as before, when it was unilaterally illuminated above the level of the cut. Boysen-Jensen was thus the first to prove the presence of a growth-promoting substance which can pass through nonliving material and still remain physiologically active.

Much excellent work has since been done on hormones, leaving no doubt that certain substances produced by the plant and others artificially applied accelerate growth; but a number of questions arise. Some substances irritate protoplasm in such a way as to stimulate development. Does it follow, then, that unless the substance is supplied, growth will not occur? In certain instances this seems to be true, but not in all. If a substance is necessary for growth, where did it come from originally? Would there not have to be a stimulus or substance to arouse the cells into hormone production? Furthermore, inquiry should not cease with the discovery of a hormone, for there yet remains to be explained the most important part of the process, namely, the physiology of the stimulation; the problem is not solved with the finding of a compound, though it is well under way.

One of the many remarkable properties of organisms is the power of regeneration, which manifests itself in plants in the production of new plants from vegetative parts of the parent, usually parts where embryonic cells are awaiting development, but also from injured regions. Scientists of a former day imagined a consciousness on the part of the plant, a "morphestesia" or "entelechy," which told it what to do. Today a growth-stimulating substance or hormone is presumed to activate the cells at the seat of the new structure.

One of the best-known examples of proliferative growth presumably activated by a hormone is that of the "life-plant," *Bryophyllum* (Fig. 57). Proliferation, however, is common in many other plants, notably certain of the ferns. The assumption is usually made that some stimulus is necessary to arouse the dormant cells, from which plantlets arise, into activity. But, if a hormone is the growth activator, what stimulus is responsible for the production of the hormone? It is often stated that detachment of a *Bryophyllum* leaf from the parent plant would bring about an upset in metabolism which might be a sufficient stimulus to initiate the formation of a hormone; and this hormone, migrating to the notches of the leaf, would cause the growth of plantlets there, provided embryonic or otherwise responsive tissue were present, which is true in *Bryophyllum*. But *Bryophyllum* may also proliferate while its leaves are attached. The stimulus, then, must be other than severance of the leaf from the parent. An unhealthy condition has been assumed to be the cause of proliferation on attached leaves; but the plant from which Fig. 57 was drawn was one of six which, though in perfect health, had all their leaves in a state of abundant regeneration.

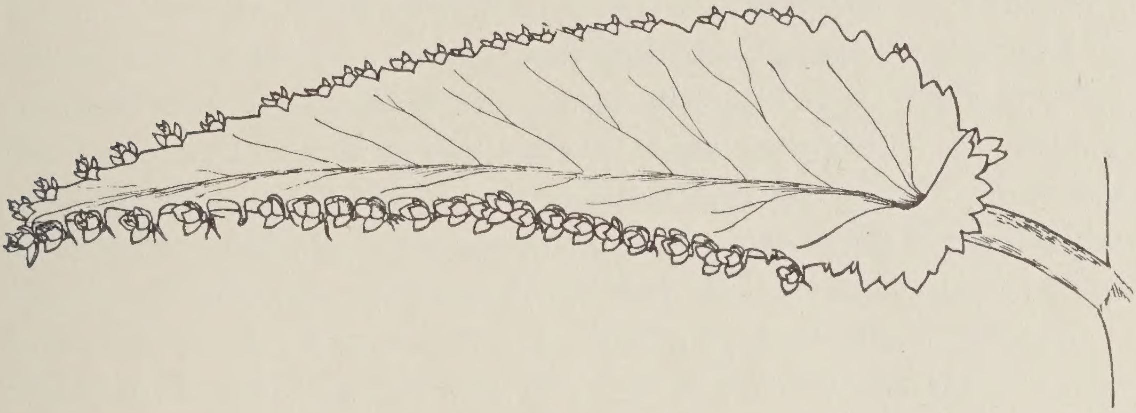

FIG. 57. Proliferation by formation of plantlets in the leaf notches of *Bryophyllum*.

Begonia leaves will sprout at any point where the veins of the leaves are cut. The leaves of *Bryophyllum* have embryonic tissue in each notch, but the leaves of *Begonia* have no such embryonic cells awaiting favorable conditions for growth. The stimulus here may be furnished by chemical substances resulting from the injury.

Nutritive substances are said to be distinguishable from hormones primarily by the quantity needed, which is minute, in the case of hormones, in comparison with the amount of food used. The difference between vitamins and hormones is less sharp, although vitamins are generally regarded as nutritive and hormones purely catalytic: vitamins feed, hormones regulate. But as the requisite amount of a vitamin is as small as the necessary quantity of a hormone, and as the manner in which vitamins function is not known, the two may be somewhat similar.

There is, however, another distinction, at least in connection with animals; namely, hormones are produced within the organism on which they act, whereas vitamins are supplied from without. The latter statement does not hold for green plants, which supply their own vitamins (page 188). Most workers prefer to distinguish sharply between hormones and vitamins.

Although the need of growth hormones and their position as distinct, specific entities have been questioned, there is no doubt but that both natural and artificial growth-stimulating substances exist.

Naturally produced growth-stimulating substances were responsible for the results obtained by Ferguson and Duggar. They found that a high percentage of germination of mushroom spores in culture was always preceded by the germination a day or two before of one or two isolated spores. When the tube of a single spore at the edge of a culture reached the central mass of spores, all began to germinate. Cultures of spores into which bits of the mycelium (fungus body) were introduced gave almost perfect germination in half the usual time. It appears that the germinating spore tube of the mycelium produces a substance that initiates growth of other spores.

Following up the work of Boysen-Jensen, Paal discovered that if the excised tip of a coleoptile is replaced off center, greater growth occurs on the side beneath the replaced tip, and curvature results (Fig. 58). In this case, illumination is not necessary.

The work so far done was sufficient to show that unequal growth resulting in curvature of a coleoptile, whether due to unilateral illumination or a displaced tip, is the result of the unequal distribution of a growth-promoting substance. This substance is produced in the tip and transported to the region of the curvature.

The entire problem was exhaustively studied in the laboratory of F. A. F. C. Went at the University of Utrecht. Here was demonstrated that the growth substance will diffuse out of the decapitated coleoptile tips if they are allowed to remain on agar blocks for two hours. Growth substances have since been obtained by diffusion into agar from numerous other plant parts. The agar blocks may then be applied to decapitated coleoptiles instead of the original tip.

F. W. Went found that auxin formed in the tip of the coleoptile is transported downwards, and while producing growth is used up, so that an auxin deficiency in the basal regions causes their growth to diminish and eventually to stop.

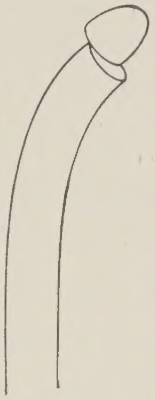

FIG. 58. Curvature due to replacing a coleoptile tip off center.

In recent years it has been shown that a variety of plant and animal material yields growth substances. Fungus cultures, maize oil, malt, yeast, human saliva, and urine are rich sources of growth hormones.

The name *auxin* has been given to the growth-stimulating hormones. Kögl was the first to isolate and identify one of its forms, *heteroauxin*, as *indoleacetic acid*. He obtained it from many sources, including urine.

Three auxins have thus far been distinguished: auxin *a* ($C_{18}H_{32}O_5$), auxin *b* ($C_{18}H_{30}O_4$), and heteroauxin ($C_{10}H_9O_2N$). Auxins *a* and *b* have not been identified in green tissue, though some plant products such as corn oil are known to contain them. The amount of heteroauxin in a root tip is very slight, but it is obtainable in large quantities from human urine, the source of many important physiological substances.

If curvature of plant parts is due to stimulation by a growth substance, then the mechanism in stems and roots must differ; for stems are negatively and roots positively geotropic, and just the reverse in their respective phototropic responses. It has, therefore, been assumed that auxin collects on the lower side of a horizontal stem, causing more active growth there and consequent upward bending, and that it accumulates on the side opposite that from which light comes, causing positive phototropic response. In the root, positive geotropic response is explained by the presence of auxin on the inner side of the curved root where, however, it *inhibits* growth, causing downward bending of the root.

The foregoing assumptions seem rather bold; first, that the same substance accelerates growth in stems but retards growth in roots and, second, that the hormone accumulates where it should to bring about the desired results. However, it has been shown by Cholodny that the growth substance from maize accelerates the growth of the coleoptiles and retards the growth of the roots, and Zimmerman and Hitchcock have demonstrated that several substances retard root elongation, yet accelerate the growth of stems. Concentration of the hormone, age of the plant, and particularly the plant part acted upon are some of the factors which determine whether a hormone will accelerate or retard growth.

Studies on hormones will probably bring about many modifications of the above statements during the next few years. Already there are signs of this, for it is being found that the problem is increasing in complexity from the physiological point of view, even with increased knowledge of the chemistry of the responsible substances. The effectiveness of auxin appears to depend as much upon successful transport in the tissue as upon the power to stimulate. F. W. Went has shown that nutrition is a controlling factor.

So long as hormones are "chemical messengers" of a complex organic nature, they satisfy the usual definition; but when the growth-activating "hormone" on the pistil of a flower is found, on analysis, to be simply the element boron, then the restricted hormone concept falls. Furthermore, it has been found that a number of substances will accomplish the same result as auxin, so that the specificity concept does not hold.

The question already put—if no growth substance is present will growth not occur?—has been in part answered by P. R. White, who found that the growing root in culture (page 240) needs certain specific substances, such as yeast extract or vitamin B, for continued development, but a growth hormone need not be added, nor can any be extracted from the roots. If growth hormones are present in young roots when cut from the plant and put into culture solutions, they will be lost quickly, yet the roots continue growing for months, even years. The supposed "essential" nature of these stimulants, therefore, is questioned by some workers.

GROWTH-STIMULATING SUBSTANCES

Growth-stimulating substances are of two kinds, naturally occurring ones produced by the plant and those which are not plant products. The naturally occurring ones are termed hormones, and under this heading they have been considered. Growth activators not produced by the plant would seem to be in quite a different category from the naturally occurring hormones, but when certain members of the two groups are found to be identical, as were auxin and indoleacetic acid, the significance of the word hormone is lessened.

The five great groups of substances—water, salts, carbohydrates, fats, and proteins—are all growth-promoting, in that all are necessary for the development of the organism.

Recent work by Zimmerman and others on substances not usually found in plants has revealed that, in addition to the specialized, naturally occurring, growth-stimulating substances such as auxin, there are many more which bring about extraordinary proliferation of plant structures; they stimulate both normal and abnormal growth.

When Kögl first isolated the growth substance, heteroauxin, he was able to obtain it in the pure state and identify it as indoleacetic acid. Hitchcock then took indoleacetic acid from another source and found that it, and a number of related compounds such as indolepropionic acid, brought about the initiation of roots, the swelling of stems, and the epinasty of leaves. Went had previously isolated a root-forming substance, *rhizocaline*, from both plant and animal material. It thus became evident that instead of there being a specific hormone for growth, root production, and other similar activities in plants, any one of a number of substances might be responsible.

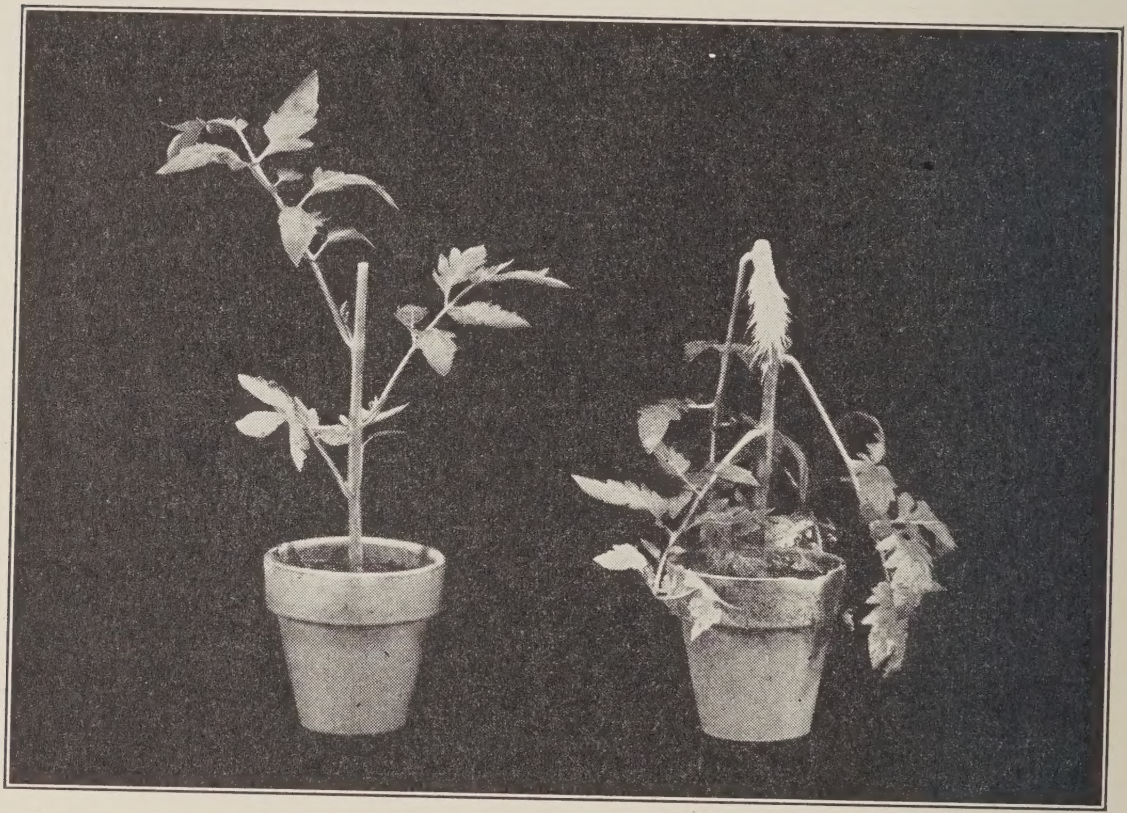

FIG. 59. Tomato plants with tops removed: left, control; right, cut surface treated with 1 per cent alpha-naphthaleneacetic acid and photographed eight days later. Note induced adventitious roots over the upper portion of the stem, and epinasty of the leaves. (Photograph, P. W. Zimmerman.)

With the foregoing viewpoint in mind, Zimmerman and Hitchcock studied the effects on plants of some sixteen substances, including carbon monoxide, ethylene, propylene, and phenylacetic acid. The principal reactions induced by these substances are local initiation of adventitious roots on stems and leaves (Fig. 59), proliferation, and other growth responses. The reactions were not always the same; thus, elongation of the sweet-pea seedling was retarded by substances which augmented growth in other plants.

The sixteen substances first studied have been added to, until now the number of known growth stimulators not obtained from plants is at least thirty-two.

Among the thirty-two acids, esters, salts, and unsaturated carbon-containing gases experimented upon, naphthaleneacetic acid and indolebutyric acid are the most successful for practical plant propagation. Fir and holly can be rooted in half the time ordinarily required for untreated cuttings (Fig. 60). Gustafson has been able to induce the fruiting of squash by treating the pistil of the flower with indolebutyric acid (Fig. 61).

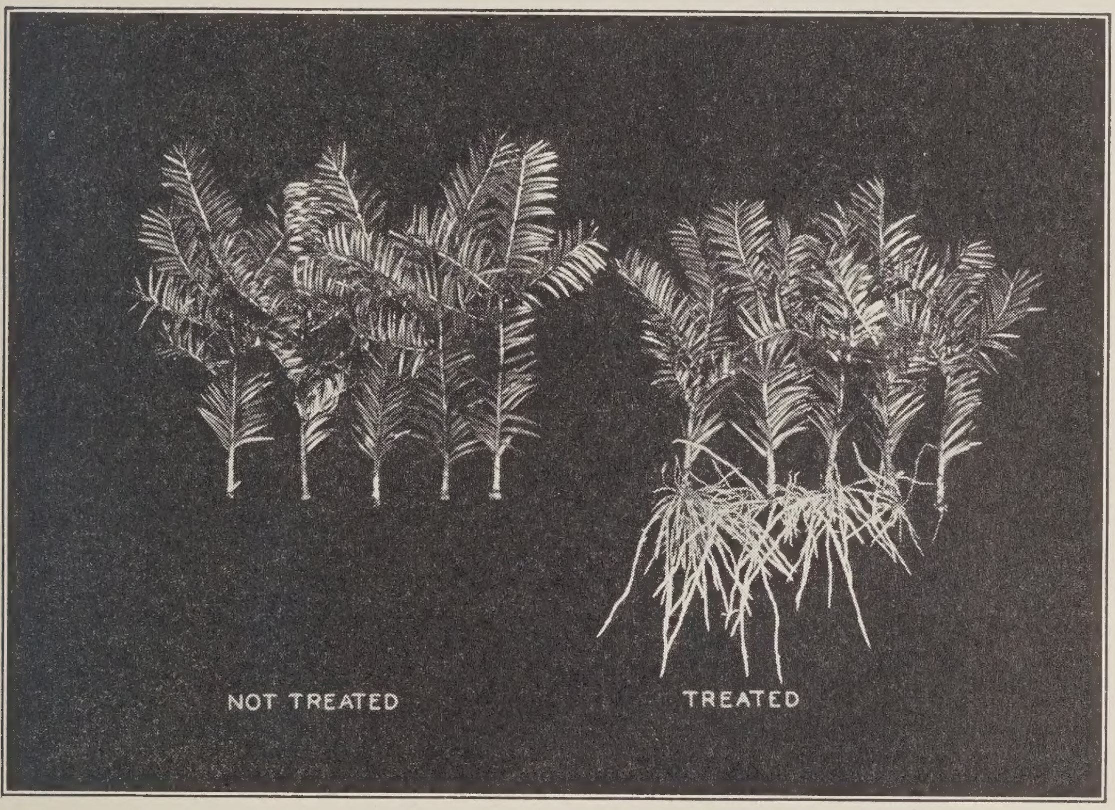

FIG. 60. Cuttings of *Taxus* treated with indolebutyric acid for twenty-four hours, then planted in the rooting medium. Photographed three months later. (Photograph, P. W. Zimmerman.)

Solutions of growth substances are effective over a wide range of concentrations, from 1 part in 5000 to less than 1 part in 100,000. A concentration of 1 part of *d*-naphthaleneacetic acid to 1,000,000 parts of lanolin will induce epinasty of tomato leaves.

Zimmerman's work makes it evident that the word "hormone" is simply a group term for many diverse substances; therefore, as he says, it is more logical to speak of the response of plants to certain chemical compounds than to the specificity of a particular growth substance. There are undoubtedly many compounds which will activate plant growth; furthermore, not all plants, nor even the same plant at all times, react the same way to any one stimulator.

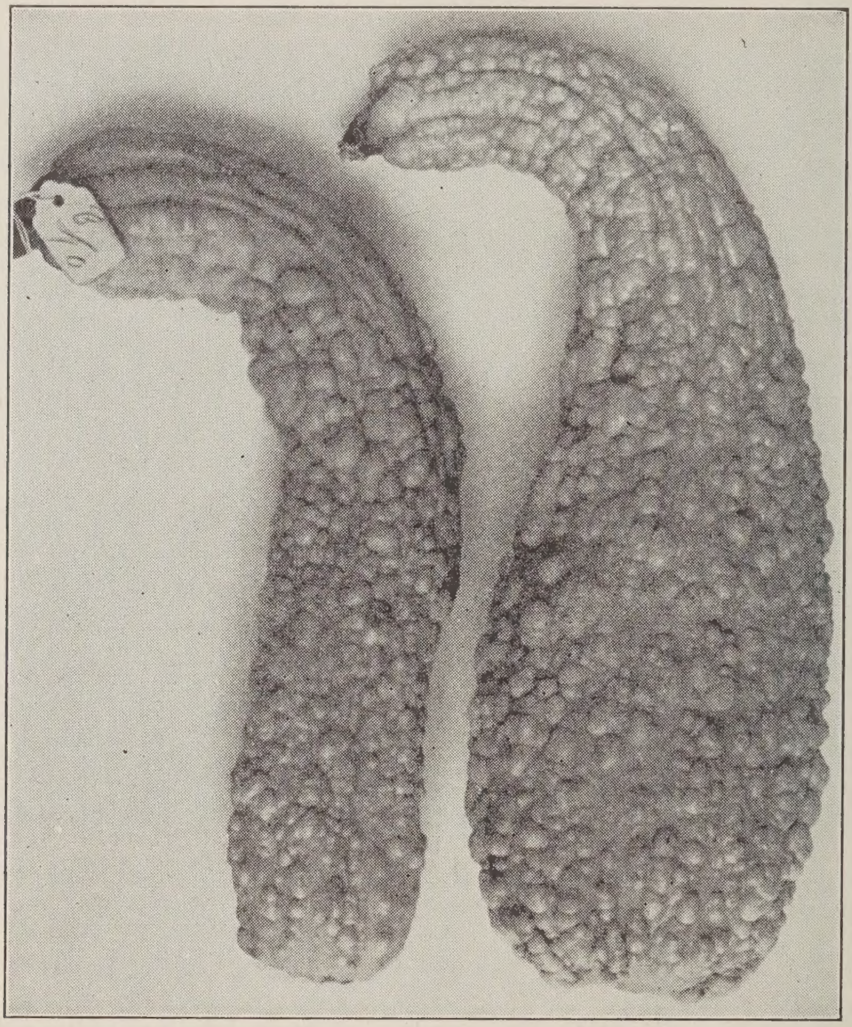

FIG. 61. Squash at left produced by treatment of pistil with indolebutyric acid, without pollination. Fruit at right is a normal one from pollination. (From F. G. Gustafson.)

Responses obtained by Zimmerman and Hitchcock include local acceleration of growth causing epinasty, or the bending of stems and leaves; systemic vs. local responses; swelling and proliferation of treated tissue; cell division and induction of adventitious roots; retardation of growth following acceleration; and retardation of root elongation followed by increase in diameter and induction of adventitious roots.

When lanolin preparations of growth substances are applied to the upper side of a tomato petiole, growth is accelerated locally and the result is downward movement of the leaf.

When the concentration is low, the growth rate is increased and the stem is caused to bend away from the treated side; but, if the concentration is high, growth is retarded and the stem bends toward the treated side.

Local application of very low concentrations induces only a local response, but if high concentrations are used, the substances are translocated and tend to induce a systemic response as shown by epinasty of leaves beyond the treated region.

Bending responses can be detected within one to two hours after the substance is applied. The activity continues and the response is complete within six to ten hours.

Proliferation and gall-like growths occur when some kinds of plants are used. These may be of the same general nature as crown galls and plant tumors. They differ, however, in that they do not continue to grow long after the substance is removed.

Five to ten days following application of the growth substance, adventitious roots make their appearance (Fig. 59). Stems, leaves, flower stalks, and young fruits can all be induced to form roots.

The responses of stem tissue and of roots are not usually the same, nor do different plants react in a like manner; thus, elongation of the sweet-pea seedling is retarded by substances which augment growth in other plants. In certain respects, however, stems and roots respond very much alike; the same substances induce adventitious roots on roots and on stems. In other respects stems and roots react differently; thus, certain growth substances cause growing stems to increase in length whereas root tissue not only does not increase in length when treated, but its growth may be retarded by the substances.

Plants in different stages of maturity and at different seasons of the year vary in their capacity to respond to treatment.

If geotropic response is due to a redistribution of natural hormones in favor of the lower side, then plants which have lost their capacity to grow upward have presumably lost their natural hormones. Such plants should make good test objects to determine if applied compounds act like hormones and might, therefore, be called "growth substances." Young tomato plants subjected to dark for three days lose their capacity to respond to gravity. When placed in a horizontal position they fail to right themselves by the fourth day. At this time the horizontal plants can be induced to turn upward or downward if treated on the lower or upper side of the stems respectively with preparations of growth substances (Fig. 62).

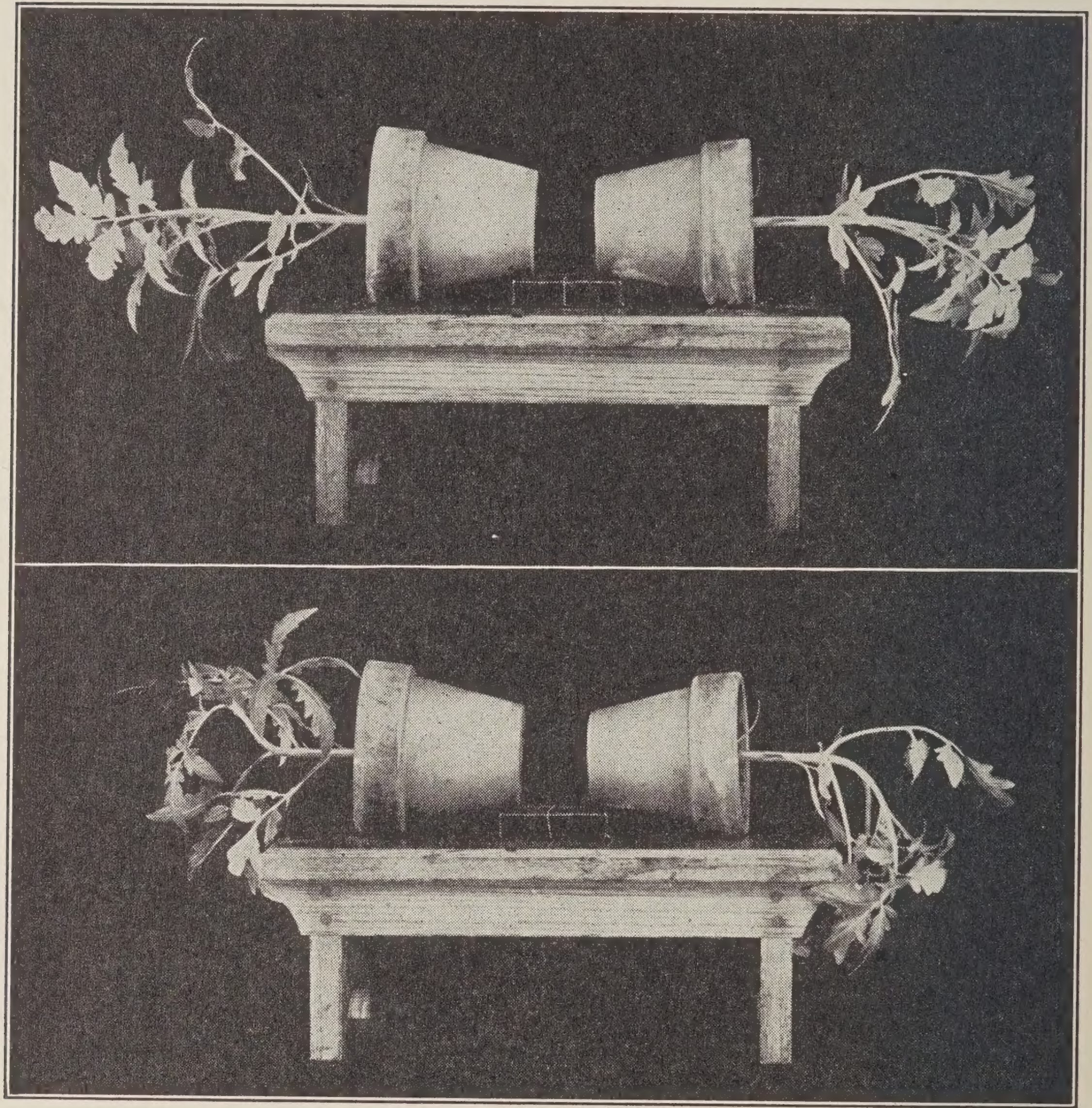

Fig. 62. Upper: tomato plants grown in the dark for three days, then placed in a horizontal position and photographed twenty-four hours later to show loss of capacity to respond to gravitation. Lower: same plants as above, twenty-four hours after treatment with 10 per cent indolepropionic acid: left, treated on under side; right, treated on upper side. (Photograph, P. W. Zimmerman.)

Among growth activators are compounds of sulphur. Hammett states that the regions of highest mitotic activity in plants, i.e., the meristems, contain a naturally occurring substance which stimulates growth, and he believes this substance to be the sulfhydryl radical, SH. This may be added experimentally in the reduced forms of parathiocresol, glutathione and the amino acid cysteine.

Pessin reports the effect of cone production on the rate of growth of vegetative shoots of long-leaf pine. He says that invariably short shoots bear staminate strobili, whereas long shoots bear pistillate strobili. He suggests that the production of staminate strobili causes a retardation in the growth of the terminal shoots, whereas the production of pistillate strobili causes a stimulation of growth of the shoots that bear them.

GROWTH INHIBITORS

It is not surprising that a seed should start growing in the spring when warmth and moisture are present, but that it should stop in the fall when in the early stages of its development is more difficult of interpretation. Once started, animal embryos and those of lower plants continue to grow to maturity; but in higher plants there is a cessation of metabolic activities and the embryo rests. When seeds are set in the fall, cold may stop growth, or weak light may do so, through lowering photosynthesis; but the cessation of development in seeds set in May or June when conditions for growth are at their best is less easily explained. There are several possible interpretations; nutrition or a growth activator may no longer be present, or a growth inhibitor may become active. The supply of nutrition in a plant which matures its seed in May or June may be diverted from the embryo into other channels; yet it is notably in the seed that food is stored. As for a growth inhibitor, if it is produced, the problem is merely pushed one step further. What arouses the tissue to the production or setting free of an inhibitor at the right moment? These questions and doubts do not disprove the theory of growth inhibitors, for such substances have been shown to exist. Indeed, the behavior of lateral buds adds additional support to the view that they are realities.

Lateral buds remain dormant while the terminal bud is intact; but, when the latter is removed, one or more of the lateral buds develop, forming upright shoots, one of which takes the place of the terminal bud. Dormancy of the lateral bud is assumed to be due to the presence of a growth-inhibiting substance maintained as long as the terminal bud is attached. It has been demonstrated that the growth inhibitor will traverse a dead portion of the stem, thus indicating that an actual substance is involved. It has also been shown that the growth inhibitor in some cases is identical with the growth-promoting substance auxin.

Although it is, at first thought, difficult to comprehend the meaning of a situation where the same substance is both an accelerator and an inhibitor of growth, yet, as pointed out in the discussion of hormones (page 212), auxin has been found to be both a stimulator and an inhibitor, depending upon the plant, the plant part, and the metabolic state of the tissue. Indeed, all the growth-promoting substances with which Zimmerman and Hitchcock worked, some thirty in number, are properly classed as accelerators under certain conditions and as inhibitors under other conditions.

As in the case of growth accelerators, growth inhibitors may also be single elements. Hammett found that the lead ion, in concentrations from 10^{-5} to 5×10^{-4}, retards the root growth of seedlings.

Although the conception of growth inhibitors has had a less favorable reception than that of growth accelerators, it is as necessary to postulate the one as the other; moreover, both the presence of growth inhibitors in plants and the fact that growth accelerators may themselves become growth inhibitors have now actually been proved.

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CHAPTER XX

GROWTH

IN GENERAL

Visual manifestations of growth are structural. Organisms become large, body forms change, cells increase in size, wounds heal, body parts are regenerated, and abnormal growths develop. These are all kinds of visual growth. From the anatomical viewpoint, growth involves simply getting big, without differentiation necessarily accompanying it.

Increase in size may come about through the taking in of water. Turgor, therefore, may be responsible for growth. The turgidity of a cell will cause distention if the walls are young and plastic, and this will result in a permanent increase in size. Growth through the acquisition of water will bring about an increase in size and *total* weight, but not in dry weight, for no solid matter is added.

Growth may also be defined as a permanent increase in weight other than through the acquisition of water, and not necessarily involving an increase in size. The increase in weight may be in the form of reserve food stuffs or, better, in the form of protoplasm; in fact, growth may be regarded as, indeed restricted to, an increase in quantity of protoplasm. However, as the maturing of cells may involve enlargement through swelling of the vacuole and consequent thinning of the protoplasmic layer without change in the quantity of protoplasm, a definition of growth restricted to increase in weight other than through the addition of water would establish the rather impossible situation of denying that growth had occurred in a plant which had increased in size.

Increase in dry weight, which is easily determined and therefore often taken as an indication of growth, is further misleading in that it may take place after a plant is mature, when growth is ordinarily regarded as complete.

Growth, as thus far interpreted, involves a change in form or an increase in bulk through the addition of water, reserve material, or protoplasm. But there is yet another kind of growth, namely, *differentiation*, which may, but need not, involve increase in weight or change in external form. Differentiation may be either structural or chemical. Structural differentiation takes place as young cells mature and acquire special functions. Chemical differentiation accompanies the maturing of cells and continues after structural change has stopped and aging has set in.

Increase in cell number, and the differentiation of cells and tissues, whether morphological or chemical, may be regarded as forms of growth which do not involve increase in size. There is, however, a strong tendency to distinguish sharply between growth and development. From this point of view, the outwardly evident changes in size, involving an increase in form, in weight, and in number of parts, constitute growth, whereas the qualitative changes which go on within the plant are developmental. Plant tumors, like animal cancers, are examples of growth not involving development. In such cases the distinction is sharp, but generally this is not so; thus, in plant meristems both growth and development occur simultaneously.

If growth is rigidly defined as an increase in size or weight, and development held to be a separate process rather than a form of growth, then a young and developing organ or organism which decreases in size or weight, must be regarded as not growing even though important embryonic changes are going on. The embryos of some animals decrease in weight and size as "growth," in the sense of maturing, continues. Children often lose in total weight while "growing" through increase in cell number, in chemical change, and in differentiation of tissues. Roots sometimes shorten as they "grow," pulling the plant more firmly into the ground. It is purely a question of terminology whether growth shall be limited to outward visible changes involving an increase in size or weight, or shall include differentiation without increase in bulk.

GROWTH REGIONS

Meristems are characteristic regions in plants where new tissues are formed. Primary meristems lie in growing points;

secondary meristems are represented by the *cambium ring*, the *phellogen* or cork cambium, and the *meristem* of leaves. Here, the production and maturing of cells take place. Growth and development of cells pass through a formative period, then a period of enlargement, and lastly a period of differentiation. Although the regions where these three periods occur may be distinguished, they are not sharply delimited, for differentiation sets in at the start.

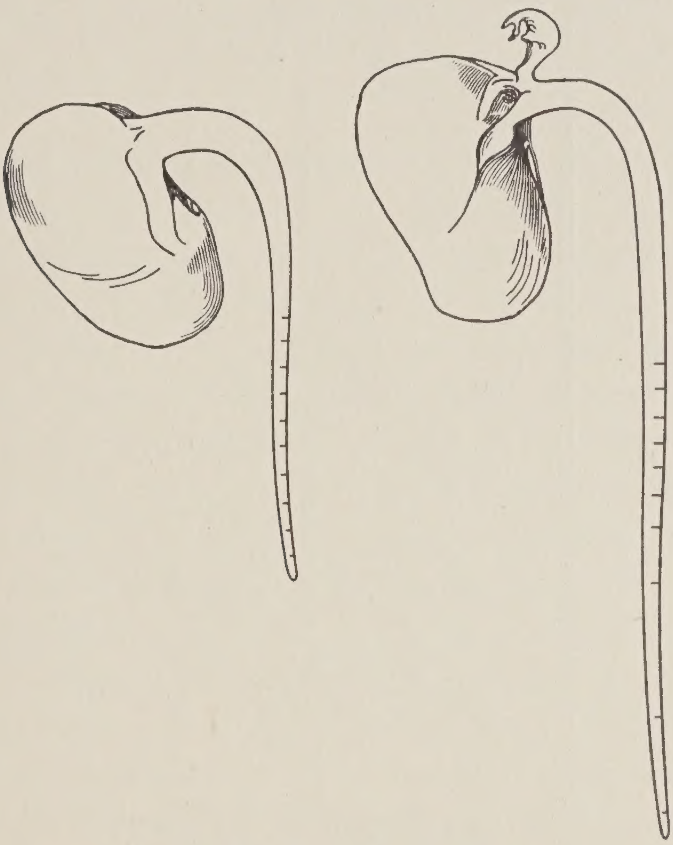

FIG. 63. Growth region in the root of a bean seedling.

The growing region of a stem or root is clearly indicated in a cut and stained section, but may be demonstrated by marking a root near its tip with a scale of dots in India ink. The root is then allowed to grow in a moist chamber and later observed. Elongation will usually be found to be greatest 3 mm from the tip (Fig. 63). In one such experiment the seedling root of *Vicia faba* showed an increment of growth for the first 10 mm as follows:

Millimeters from tip	1	2	3	4	5	6	7	8	9	10
Growth increment	1.5	5.8	8.2	3.5	1.6	1.3	0.5	0.3	0.2	0.1

MEASUREMENTS

Ways of measuring growth are numerous and vary according to the particular property which has been chosen as the standard for measurement, whether increase in height, diameter, total weight, or weight of dry matter. Measurement of the height of the plant at the beginning and the end of the experiment is the simplest way. Accurate measurements of this sort can be made

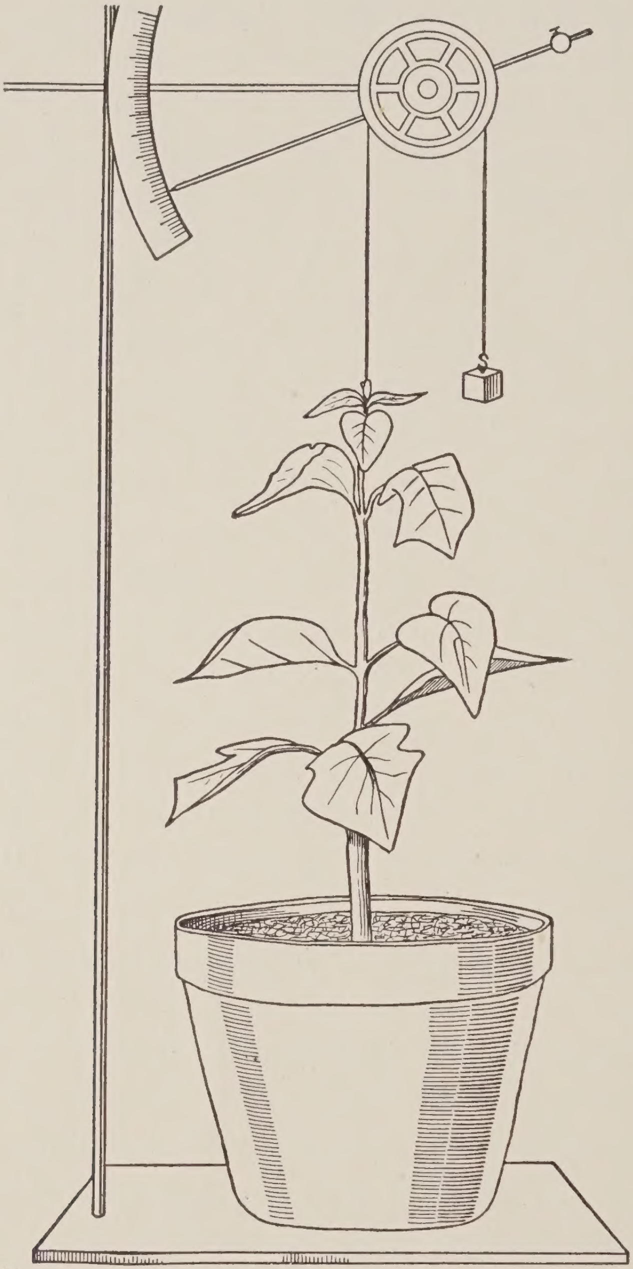

FIG. 64. Auxanometer for measuring growth.

by using a horizontal microscope and recording the height of the plant day by day, or by using an *auxanometer*, which in its elementary form consists of a thread, a pulley, a pointer, and a curved scale (Fig. 64). An auxanometer becomes self-recording by allowing the pointer to rest upon smoked paper held on a revolving drum; the elongation recorded is then a time-rate curve. Other forms of auxanometers include models designed to measure the increase in diameter of trees.

RATE

Rates of growth differ greatly. Extremes are of especial interest. Among fast growers are herbaceous annuals which attain maximum heights of 10 to 12 feet in a summer. Corn (*Zea mays*) and the giant *Heracleum* of the Caucasus reach 12 to 15 feet in a summer. If 10 feet is reached in 2 months, this will mean 2 inches a day. The actual maximum rate is greatly in excess of this, for the growth of plants is at first slow, then increases rapidly, and finally slows down to zero. Among the fastest growers are bamboos; 7 m 85 cm in 3 days is recorded. The "kudzu" vine, *Pueraria thunbergiana*, grows as much as 12 inches in 24 hours. The slowest growers are trees which attain great age. During an unfavorable season the increase in the radius of a large tree may be so slight that the annual rings are not far enough apart to permit counting them without the aid of a lens; as many as 10 years' growth may be crowded into one-eighth of an inch with only two or three layers of cells per year.

E. C. Miller has expressed the daily increment of growth in terms of percentage increase in dry weight. *Zea mays*, planted May 25, reached its maximum rate of increase within 14 days. During the next week the growth fell as rapidly as it rose, and continued to fall gradually. Gain in total dry weight, however, increased constantly.

Attempts have been made to show that growth follows a definite law which can be expressed mathematically in terms of the initial dry weight, the final dry weight, the time, and a constant or two, but all the formulae rest upon data insufficient to permit regarding any one of them as a true mathematical expression of growth.

POLARITY

Few properties of living matter are more significant and more widespread, and yet have received so little attention as polarity. It manifests itself in plants strikingly in such an experiment as the following. If a willow cutting is placed in the ground with that end uppermost which was uppermost on the parent tree, it will grow roots at the lower end, in the soil, and send out young shoots from the upper end, which is as it should be (Fig. 65A). But if a willow stick is put into the ground bottom end up, roots will grow above the leafy shoots further away from the ground than the young shoots (Fig. 65B). The roots turn downward obeying the law of gravitational attraction, and the

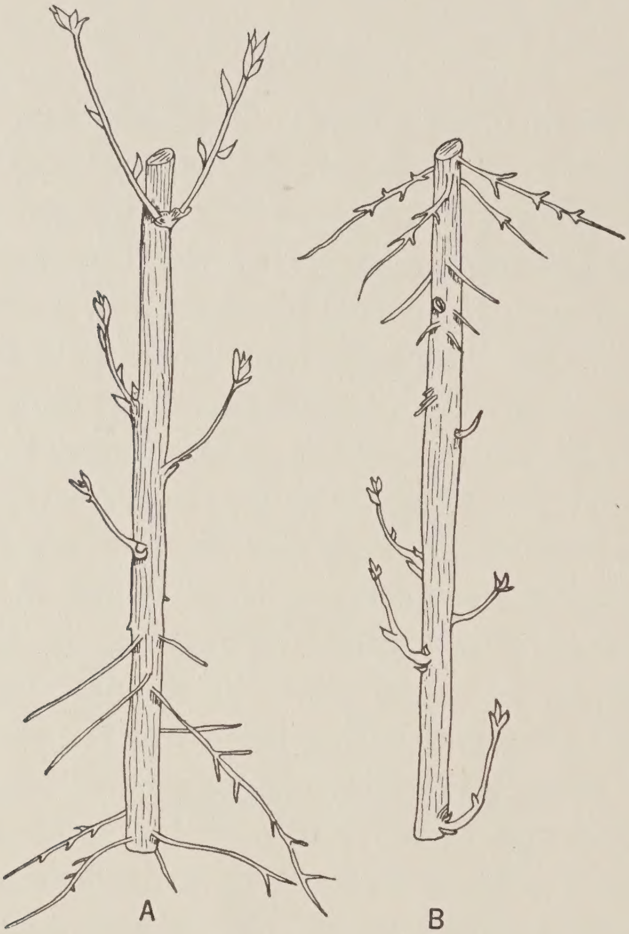

FIG. 65. A. The polarity of a willow twig; stems above; roots below. B. The polarity of a reversed willow twig; roots above but pointing downward; stems below but pointing up. Original polarity is maintained but newly developed organs respond normally to gravity. (After Vöchting.)

stems turn upward, but each is at the wrong end of the branch for proper growing. The cause of this is a previous polarity in the twig established when the twig was a part of the parent tree.

Not only do plants as a whole and their stems show polarity, but leaves, fruits, and individual cells do so as well. F. Weber has illustrated polarity in cells by demonstrating that they all plasmolyze at the same end first.

Plant physiologists, writing on polarity fifty years ago, debated the possibility of it being due to vital forces, so remarkable a quality of living matter was it thought to be. But the only scientific explanation is a mechanistic one. The plant and its parts have acquired this property through physical forces, such as gravity, working upon them during growth. The force need not be gravity, thus concentration gradients established as a result of the upward movement of solutions from the soil, may be responsible. The polarity may also be an electrical one, the upper part positive in relation to the negative vegetative basal region.

Polarity is a very fundamental property of all matter being characteristic of molecules such as those of amino acids. It may be that molecular polarity is, through molecular orientation, in part responsible for the polarity of plants.

FACTORS INFLUENCING GROWTH

Factors affecting growth rate are many. For most tissues, organs, and plants, a certain amount of light is necessary for normal growth. Intense light may, however, retard growth. Thus Balls has demonstrated a retarding sunshine effect on the growth of cotton and other plants in Egypt where the light is particularly strong. Increase in thickness of fiber has been observed to take place primarily at night in Egyptian cotton.

Anderson has shown that the normal growth rhythm in cotton fiber is abolished by continuous illumination (page 292, Fig. 92).

Temperature augments growth, but the optimum depends upon the kind of plant and the length of time applied. It is particularly important that corn should have hot, moist nights, such as those for which the corn-growing areas of America are noted. Most ferns, on the other hand, "like" a cool environment, and with it moisture. This is why they are usually found in the shade, which has led to the belief that they "prefer" shade.

Certain ferns, such as the temperate hay-scented fern *Dennstaedtia* and the tropical *Gleichenia*, can tolerate dryness and are found in hot, exposed, and sunny areas, where they flourish. The tree fern illustrates the preferred situation of most ferns; a well-developed specimen always has its crown fully spread in the open where there is bright sunlight, but its habitat is a cool ravine with its roots in wet soil.

Increase in temperature, up to 30°C, will usually accelerate the growth of plants when first applied; but if high temperatures which are at first beneficial are too long applied, retardation and other detrimental effects follow.

Plants tolerate extreme temperatures to an extraordinary degree. The temperature of plants, like that of cold-blooded animals, rises and falls with the temperature of the environment. A cornstalk in the sun on a hot summer's day may reach 37°C (100°F); its temperature then rises very slowly, lagging behind that of the air. Under severe conditions a corn plant may be burned by the sun. Temperatures of 55°C have been recorded for cacti in the Arizona desert. The blue-green alga, *Oscillatoria*, grows luxuriantly in the water of hot springs on the slopes of volcanoes at a temperature of 57°C (135°F). Few records have been kept of minimum plant temperatures, but they are at least known to fall below freezing (0°C). The protoplasm, however, may not freeze, either because of dissolved salts and sugars which lower the freezing point, or because the water is colloidally bound (see page 91).

Recent work by Luyet tends to show that protoplasm dies if its temperature is gradually lowered to below the freezing point; yet if tissue is plunged into liquid air, though immediately frozen, it later recovers.

The factors influencing growth thus far considered are environmental ones which the plant is likely to meet in its natural surroundings. There are also external factors, or growth regulators, which may be artificially applied, and internal factors produced by the plant. These latter two are considered in detail under the chapter on hormones and growth regulators. The artificially applied growth substances are of interest only in so far as they may prove of practical importance (Fig. 60) and may throw light on the physiology of the naturally occurring ones.

Studies on growth regulators indicate that growth responses involve a chain of essential compounds; if any one is missing there can be no growth. Plants growing in the dark for several days lose their natural hormone which controls geotropic response, and therefore they cannot right themselves when placed in a horizontal position. An artificially applied growth substance supplies the missing link in the chain (Fig. 62).

DORMANCY

Dormancy follows a cessation of growth, life persisting, but with a suppression of metabolic activities. It is a common quality of plants. All perennial plants are dormant in winter in temperate regions. Seeds are dormant and some remain in that condition for many years. This raises the interesting question, do any metabolic processes, in particular respiration, continue throughout this time (see page 157)?

Attempts to disturb dormancy and thus alter the life cycle of organisms, have led to important theoretical and practical results in hastening plant development and accelerating the maturing of fruits.

Among the early efforts to alter the life cycle of plants is that of Klebs, who undertook to shorten the winter rest of trees. He brought various species into the greenhouse in early fall and sought to end their dormancy with warmth and moisture. He found that some species would come into leaf within a few weeks' time; others could not be aroused until after several months of rest; and still others defied all efforts to arouse them until the arrival of early spring when the normal time of rest had passed.

Freezing, drying, and the application of ether vapor are the most effective stimuli for breaking the rest periods of perennials. Some plants do not assume normal growth in the spring unless they have been subjected to chilling, i.e., to winter weather.

F. E. Denny has shown that the gas ethylene, or, still better, ethylene chlorhydrin, will overcome dormancy; its use has also been found to be one of the best means for ripening fruits. R. B. Harvey has interpreted the ripening effect of ethylene as due to the activation of enzymes which convert starch into sugar and oxidize acids and tannins.

PHOTOPERIODISM

The physiology of growth is an introduction to the physiology of reproduction. Any process which will hasten growth, and thus cause a plant to go through the successive stages of its life cycle more rapidly, also hastens reproduction. This is shown to be true in the process known as *photoperiodism*, which involves a disturbance of the usual life span of plants through changes in illumination.

Klebs, in his general studies on the breaking of dormancy, found that both prolonged vegetative growth and premature flowering were induced by abnormally long or short exposures to light, temperature also being a factor. The developmental steps in the life cycle of a plant could thus be greatly hastened or retarded for a surprisingly long time. Klebs attributed the acceleration to the accumulation of carbohydrates, but he thereby merely pushed the problem one step further back, for why do the carbohydrates accumulate? Excess in carbohydrates is just as likely to be the result as the cause of hastened growth and premature flowering.

As photoperiodism bears directly on the problem of reproduction in plants, and the effects of environmental influences, it will be considered again under these headings with special reference to the work of Garner and Allard (pages 254, 288).

VERNALIZATION

Vernalization is the treatment of seeds by methods which will hasten flowering. It thus affects growth, but is best considered in detail under reproduction (page 254).

RHYTHM

Rhythm characterizes nearly all natural phenomena. Day and night; seasons; tides; the life of an organism—its birth, growth, senescence, and death; the functioning of body parts, such as the beat of the heart and heat in animals; the making, storing, and using of sugar by the plant; and flowering in plants—all are rhythmic.

Many rhythmic processes are so familiar that they are not regarded as extraordinary, but it is only necessary to look a little farther to find other examples which are not so familiar, nor so easily interpreted. For example, the germination, growth, flowering, fruiting, and death of annual plants coincide with the seasonal cycle so perfectly that it is quite clear why seeds germinate in the spring, plants flower and fruit in the summer, and die in the fall.

Growth rhythms often coincide with seasonal or daily or other meteorological cycles. Trees show by their *annual rings* that their growth rhythm is a yearly one; whereas the cotton fiber shows by its rings or lamellae (Fig. 92) that its growth rhythm is diurnal, for 21 rings correspond to the 3 weeks of the growing period (page 227). The interpretation of such growth cycles is not difficult. Less easy of explanation are some of the longer sexual rhythms in plants. Certain bamboos and palms live many years, 32 in the case of bamboos and 40 in the case of some palms, without reproducing; they then flower, mature their seed, and die. The phenomenon is surprising enough when met with in an individual plant, but becomes startling when the sexual cycle holds true for all the plants of one kind throughout an area covering several square miles. The climbing bamboo, *Chusquea*, flowered and then died throughout the mountains of Jamaica in 1918, 32 years after their previous flowering in 1886. Several years earlier Joseph Hooker had taken two specimens back to Kew Gardens in England, and, on hearing by letter that this climbing bamboo had come into flower throughout the mountains of Jamaica, hurried to examine his specimens in the greenhouse; they, too, were in flower.

In India and Burma, a 32-year life cycle is quite common among the bamboos. The years in which the gregarious flowering of bamboos occurred in parts of China have been recorded on ancient manuscripts, and each date is 32 years, or a multiple of 32 years, from the previous one.

The talipot palm, *Corypha*, whether growing wild or planted in gardens, often exhibits striking gregarious flowering, as it does in Ceylon, where an avenue of these plants flowered simultaneously for the first time after 40 years' growth.

The gregarious flowering of many species over large areas has been attributed to drought, but this was not the cause in the case of the Jamaican bamboo or of the talipot palm of Ceylon. Severe drought often occurs with no flowering and flowering with no drought. Climatic conditions could not possibly be the cause of the simultaneous flowering of climbing bamboos in Jamaica and England, 4000 miles apart.

The destiny of an egg is determined by two kinds of factors, heritable, or innate ones, and environmental ones. The innate factors are part of the protoplasm. Geneticists have given the name *gene* to certain of the constituents or qualities of protoplasm which are transmitted from generation to generation. Environment plays its part and often an important part in the destiny of an egg, but the germ plasm determines whether an egg will grow into a frog or a tree. That the life cycle of an organism is determined primarily by innate heritable qualities is evident from the fact that the span of life of organisms differs greatly among species, yet is more or less constant among the individuals of a species, the environment being the same. Plants possess an innate heritable character which determines, under natural conditions, the age at which they attain sexual maturity. When a group of plants flower and die together, they do so because all are of the same age, having been sown from seed at the same time when their parents flowered gregariously.

Though certain life cycles appear to be due to a protoplasmic quality, the conclusion can hardly be escaped that seasonal growth rhythms were originally determined by the seasonal cycle. Longer cycles such as the 32- and 40-year sexual rhythms of bamboos and palms may have been established by climate, although there is no conclusive evidence for it. However, some laymen and scientists give support to this possibility. Thus Francis Bacon wrote:

> "There is a toy, which I have heard, and I would not have it given over, but waited upon a little. They say it is observed in Low Countries (I know not in what part) that every five

> and thirty years the same kind and suit of years and weathers come about again; as great frosts, great wet, great drought, warm winters, summers with little heat, and the like, and they call it the prime; it is a thing I do the rather mention, because, computing backwards, I have found some concurrence."

The sexual cycle of some plants so perfectly synchronizes with rhythmic geological phenomena that a definite relationship probably exists, as in the case of the brown alga, *Dictyota*, which discharges its eggs and sperm precisely at spring tides (page 252).

The problem is one requiring much investigation, but of plants it can be said that life cycles, including sexual periodicity and the seasonal rhythms, are well established; the environment merely determines when each stage in the cycle is to begin and end. This is illustrated in a small apple tree planted at Tjibodas in Java, where there is no winter season, nor even the dry and wet seasons common to the tropics. This tree, having come from Holland, was accustomed to a seasonal cycle of foliation and defoliation, of flower and fruit production. The tendency to observe this rhythm was there, even though there was in Java no climatic cycle for timing the rhythm. The result was that in July one limb was bare, one in full foliage, another in bud, and still another with fruit; the innate rhythm continued but was completely disarranged.

The factors underlying rhythm in nature are fundamental, but they are capable of being disturbed in certain instances. The work of Klebs has been mentioned (page 229) and the work of Garner and Allard on hastening and retarding the time of flowering by decreasing or increasing the amount of illumination will be considered (page 288). However, although an environmental factor may influence a phenomenon, it cannot for this reason be regarded as the cause. Thus, Garner and Allard have proved that length of exposure to light determines the time of flowering in certain plants, but the cause of the flowering lies in the physiological mechanism of the plant.

THE SPIRAL HABIT

There is a pronounced tendency in organisms to grow and move spirally. Tree trunks are often spirally twisted. The wind was supposed to be instrumental in this until it was noticed that roots, which the wind cannot reach, also show spiral twisting. The stems of twiners and tendrils are spiral. Many species of climbing plants twine persistently to the right, as does *Convolvulus arvensis*, or to the left, as does *Humulus lupulus*, but some may twine in either direction and some change frequently from right to left and left to right (Fig. 66).

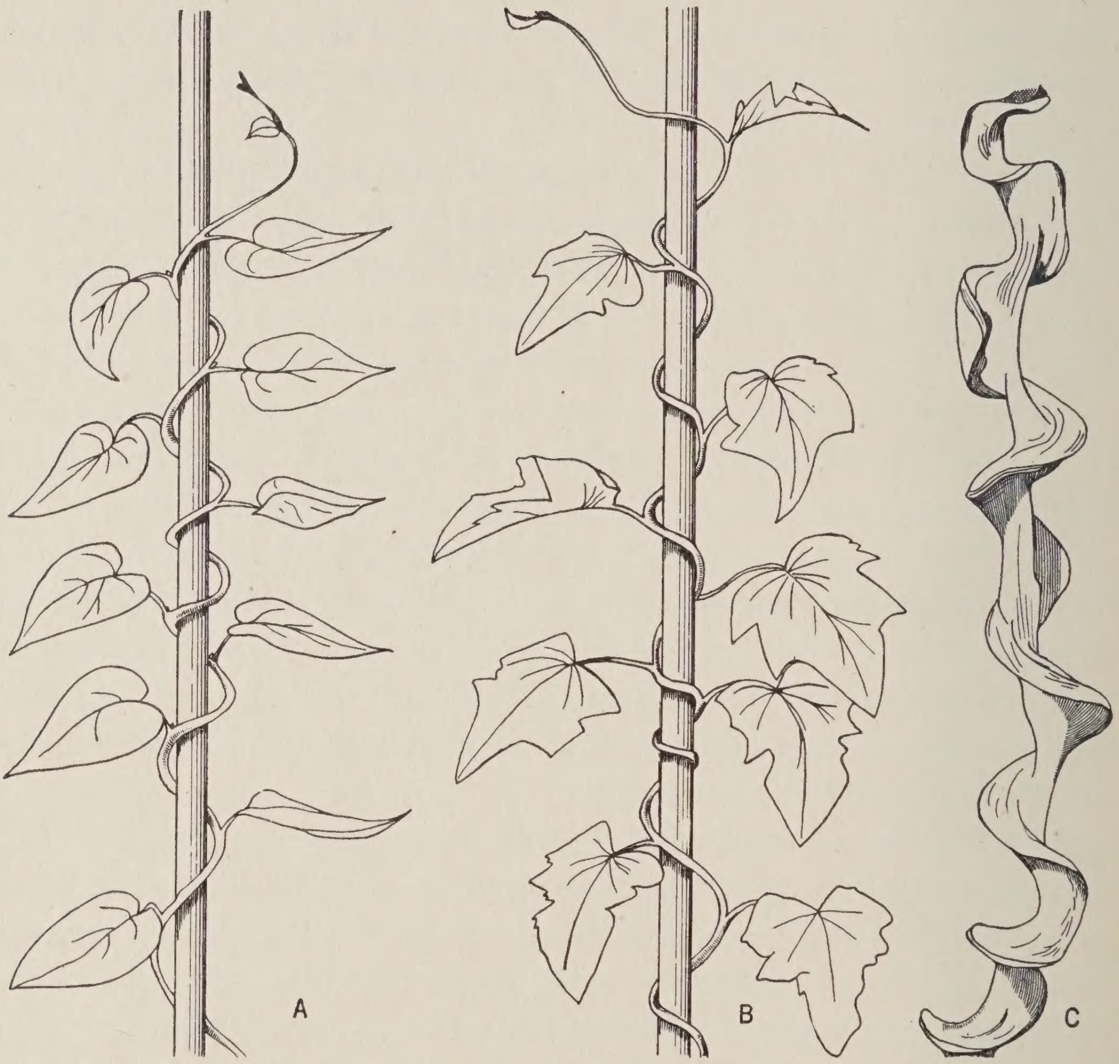

FIG. 66. A. Left-hand twiner. B. Right-hand twiner. C. Reversed spirality in the tropical liane, *Bauhinia*, five reversals within three feet.

Charles Darwin made many meticulous observations on the twining of plants. He observed no case of two species from the same genus climbing in opposite directions, though such a case is reported for a South American relative of a European species. It would be of value to know if this difference is due to the fact that the situations of the two species are in different hemispheres, which is the explanation for left-hand and right-hand whirlpools.

Occasionally, different individuals of the same species twine in opposite directions. This behavior is rare among species but if characteristic of a species is not uncommon among the individuals of that species. The same is true for reversal in the direction of twining; it is not common, yet may frequently occur within a given species. Darwin reports reversal in *Ipomoea.* Fig. 66C shows five reversals within a length of 3 feet of the oriental monkey-vine, *Bauhinia.*

Both the larger and the smaller members of the cellulose framework of plants twist, for cotton fibers and bast fibers show spiral growth. The walls of bast fibers are built of spirally wound fibers lying in successive layers (Fig. 52).

Spiral growth in plants is further illustrated in the orientation of leaves where helical arrangement is common and can be represented by mathematical formulae. E. S. Castle has demonstrated spiral growth in Phycomycetes and shown that the rotation of sporangia takes place at a constant rate, occurring in the region of growth or elongation (Fig. 67). W. D. Francis says protoplasmic constituents show spirality.

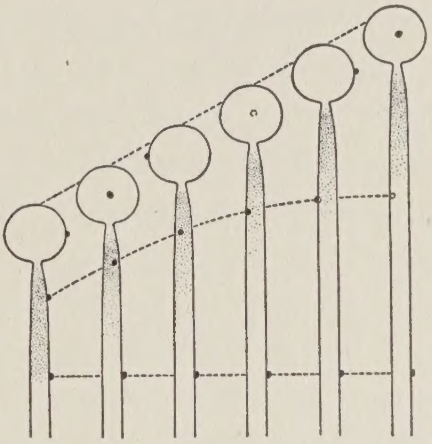

FIG. 67. Spiral growth of the sporangium of *Rhizopus*: the rotation of the sporangium and its stalk is shown by the positions of the attached particles. (From E. S. Castle.)

The spiral habit is also widely spread in animals. Sea snails (*Nautilus*) develop spiral shells, which, like trees, show both right or *dextral* and, less often, left or *sinistral* rotation. Muscle fibers are often twisted. Movements in organisms, from ameba to man, show a spiral trend. The list of examples could be increased to include objects as small as chromosomes, which are now known to be spiral, and as large as spiral nebulae, which, like the spiral of Archimedes, can be mathematically expressed. Although a hunt for spirals yields a motley crowd, the individuals of which appear to bear no relationship to each other, there may yet be one fundamental cause for many spiral forms in nature.

The cause of spirality is not known in most cases, though there is a convincing explanation of right and left rotation in whirlpools. Whirlpools rotate counterclockwise north of the equator and clockwise south of the equator. That side of the whirlpool nearest to the equator is dragged in the direction of the earth's rotation, which is west to east; hence, the southern side of a northern hemisphere whirlpool and the northern side of a southern hemisphere whirlpool move west to east; thus is the direction of rotation determined.

The earth's rotation may not be the determining factor of spirality in organisms, for there are both left- and right-hand twists in most species. Unequal growth is the ultimate cause of spirality in many plant parts. Plant and animal organs do not always grow equally on both sides; any deviation from a straight line, no matter how slight, will result in a spiral.

A more fundamental interpretation of spirality in organisms is that which ascribes to protoplasm a tendency toward right- or left-handedness. Just what this means will become clear in the presentation of the remarkable findings of Pasteur on the capacity of protoplasm to distinguish and select levo- from dextro-tartaric acid (page 239).

SYMMETRY AND HARMONY

Symmetry in growth and development manifests itself in various ways and is a form of rhythm apparent in *harmony.*

A physical explanation of *symmetry* in organisms—whether it is the bilateral symmetry of man or the orderly arrangement of leaves on plants—has been sought in the laws governing crystal growth. Symmetry and an *orderly asymmetry* are properties of both living matter and crystals.

Geometrical crystallography is based on two fundamental laws, the constancy of angles and the rationality of intercepts. The first states that at a fixed temperature homologous interfacial angles on all the crystals of any one chemical compound are constant, regardless of the size or shape of the crystal. The second involves the positions of faces on crystals. A set of three, or in certain cases four, straight lines, termed *crystallographic axes*, intersecting at a central point, is assumed to pass through the crystal body. The several faces are projected until they intercept one or more of these axes. The law of the rationality of intercepts states that on any crystal the ratio between the intercepts of one face on two or more such axes bears a simple rational relation to the corresponding ratio of intercepts of any other face. When the intercept ratios of the faces in a zone, that is, in a series with mutually parallel intersections, are determined, they will be found to bear simple multiple relations, the multiplying factors most frequently observed being, in order: 2; 3/2 and 3; then 4/3, 5/3, 5/2, and 4.

Harmony is convincing evidence of the presence throughout nature of a uniform law of the distribution of parts. The crystallographer, Victor Goldschmidt, evolved a mathematical system of harmony which shows remarkable applicability to natural phenomena. The crystallographic law of rationality of intercepts and indices discussed in the preceding paragraph has to do with the mathematical relationship of the distances between nodes. If values are taken between three important nodes, namely, 0 (the origin of measurement), 1 (the unit of spacing), and ∞ it will be found that, when there are two additional nodes of the zone segment, these occur generally at the distances 1/2 and 2; when there are still more, they tend to lie at 1/3, 2/3, 3/2, 3, and so on. That is to say, of the many rational spacings which might occur, certain simple proportional relationships are preferred. Goldschmidt thought the crystallographic number series might be of fundamental importance not only to crystal structure but also to other natural phenomena, so he applied them first to music. He transformed the relative vibration periods—1, 9/8, 5/4, 4/3, 3/2, 5/3, 15/8, 2—of the notes of the major scale—C, D, E, F, G, A, B, C—into the form 0 - - - ∞, and he obtained a series of numbers similar to the crystal number series. He then proceeded to carry the system of harmonic analysis to other fields. By selecting the most prominent of the Fraunhofer lines of the solar spectrum—A (purple), B (scarlet), C (red), D (yellow), etc.—he transformed the relative wave lengths into the form O - - - ∞, and again obtained the same number series. Finally, turning his attention to cosmic space, Goldschmidt found that the distances of the planets and of the asteroids from the sun—which had been mathematically expressed by the now discarded law of Titus and Bode—conform to the law of harmonic analysis when transformed by the same method used for the music and color scales. The satellites of Saturn, as also the seven hundred or more asteroids, fall into groups at distances from the central object—planet or sun—corresponding to the crystallographic series. Goldschmidt predicted that, if another planet existed, it would lie at the position 4 in the series. He lived to see this speculation accurately substantiated by the position of the newly discovered planet, Pluto. The Goldschmidt series of the positions of the major planets thus becomes:

SUN	JUPITER	SATURN	URANUS	NEPTUNE	PLUTO	OUTER SPACE
0	½	1	2	3	4	∞

M. A. Peacock calls attention to the ancient belief in an harmonious universe. Pythagoras knew the numerical relations of the vibration periods of musical intervals, and, convinced that "number is the essence of things," he dreamed of a universal harmony which could be stated in rational numbers. Ptolemy, and after him Kepler, sought for harmony in the motion of the planets.

Recognizing, then, a universal harmony, which can be expressed as a definite law of rational numbers, one may consider the possibility of applying this law to processes in the living world. It is a difficult task, but Goldschmidt attempted it, as have others in one form or another.

The most convincing proof that there is in living matter an orderly arrangement of parts which is specific and crystallographic in character is to be had from the remarkable discovery of Pasteur which laid the foundation of the science of *stereochemistry*. Not only is the symmetry, or asymmetry, of protoplasmic organization crystallographic, but it exhibits spirality.

Pasteur noticed that the crystals of tartaric acid and the tartrates had minute faces. These faces beveled only one half the edges, distributed in such a way that a mirror image of the crystal could not be superimposed on the crystal itself. Pasteur reasoned that this external dissymmetry of form foretold internal or molecular dissymmetry. The tartrate which presented these faces, or dissymmetry, caused the plane of polarization to deviate to the right. Paratartrate, on the other hand, was optically neutral. Pasteur expected to find it lacking in dissymmetry, but found that some of the paratartrate crystals had faces inclined to the right and some had faces inclined to the left. He then separated the two kinds of crystals and made solutions of each. Observing them in the polarizing apparatus, he found that the two solutions gave contrary deviations, and a mixture of equal quantities of each had no action on light. Thus arose stereochemistry.

A most interesting feature of this work was the discovery that, as Pasteur expressed it, "If I place one of the salts of racemic acid, paratartrate, or racemate of ammonia, in the ordinary conditions of fermentation, the dextrotartaric acid alone ferments, the other remains in the liquor." Pasteur points out that this is the best means of preparing levotartaric acid, and adds that the selection by the ferment takes place because the ferments "feed" more easily on the right than on the left molecules. Later, Pasteur grew the green mold, *Penicillium glaucum,* on ashes soaked in paratartaric acid, which, as already noted, is a mixture of dextro- and levotartaric acid, and found that the levotartaric acid disappears, because it has been consumed in "preference" to the dextrotartaric acid. Whether this selection between dextro- and levorotary molecules is to be attributed to ferments or to a living organism is unimportant. In either case, a ferment is responsible whether added separately or secreted by protoplasm.

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CHAPTER XXI

PLANT CULTURES

Studies in growth, nutrition, reproduction, and related processes have led to the development of methods in investigation which have yielded some significant results which bear on many diverse problems in physiology. They are, therefore, assembled here in a chapter on methods in plant culture, though they would fit equally well under the chapters on salt requirements, foods, growth, or other subjects in which field they were developed.

TISSUE CULTURE

Cytology, or the science of cellular study, has been greatly enriched through the technique known as *tissue culture*, which had its beginning in 1902 in some unsuccessful attempts by Haberlandt to grow isolated bits of plant tissue in culture media. Where he failed with plant material, Ross Harrison succeeded with the more easily handled animal tissue. The work was then taken up by Carrel in America and Strangeways in England. It has led to some very significant findings, the most dramatic of which is the continued culturing of a small piece of chick heart for twenty-six years. Although none of the original tissue now exists, the progeny of the original cells is still alive.

Unfortunately, it has not been possible to culture plant cells as wholly undifferentiated tissue, but a very near approach to this has been accomplished by Robbins, Kotte, Gautheret, and Philip R. White in the culturing of root tips.

Before root culture is taken up, brief mention should be made of some attempts, notably by C. Hock, to grow isolated protoplasts. The living protoplast of *Elodea* can be removed from the cell wall after the cells have been slowly plasmolyzed in sugar solutions of increasing concentrations. Following plasmolysis, the tip of the cell wall is cut off, whereupon the protoplast may flow out into the sugar solution and assume a spherical shape (Fig. 68). Protoplasts obtained in this manner were kept alive by Hock for more than thirty hours, during which time protoplasmic streaming continued.

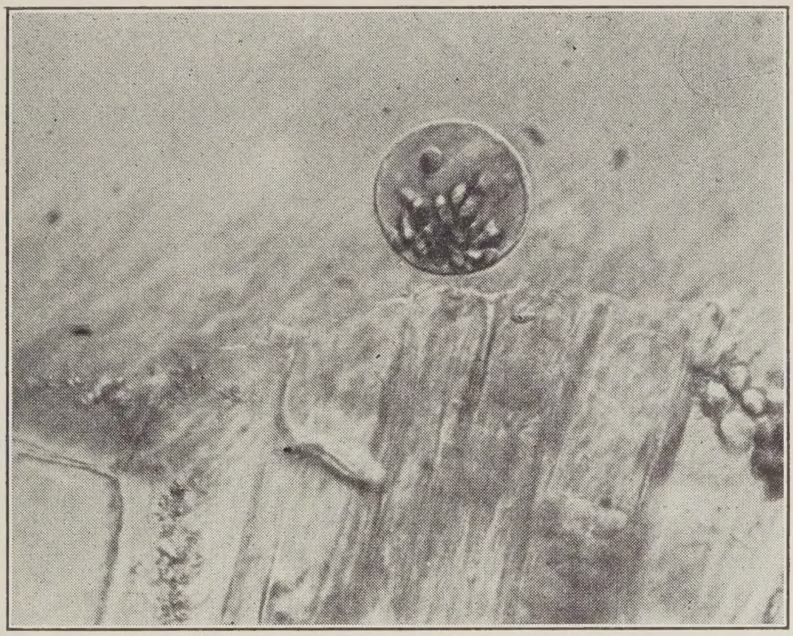

FIG. 68. A freed protoplast. (Photograph, Charles Hock.)

The root culture work of Philip R. White is the most extensive and successful yet done. He has kept roots actively growing for four years, and they are still growing at the time of this writing (Fig. 69). The tip of a young root is cut off and put into a suitable culture solution. It grows actively and forms side roots; but

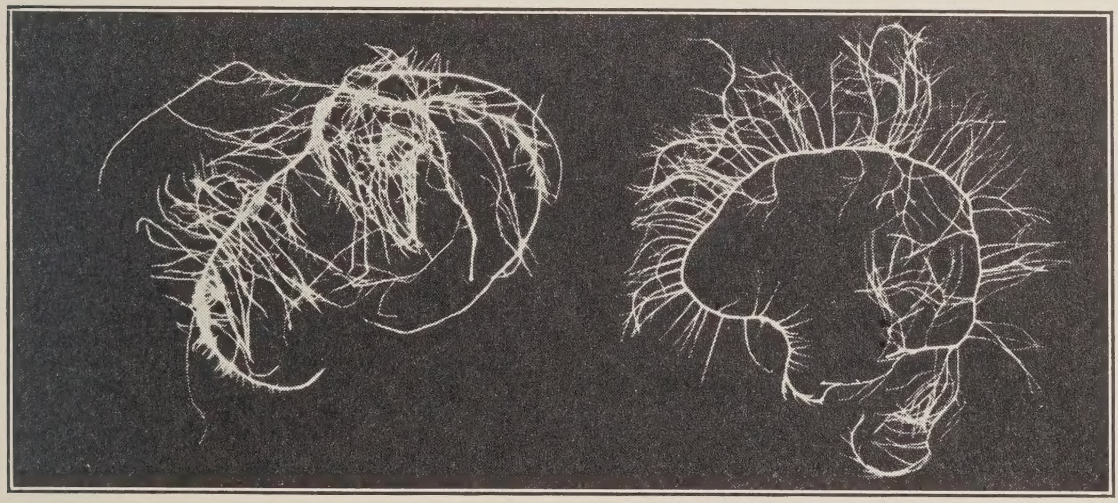

FIG. 69. Cultures of excised roots: left, *Petunia;* right, *Trifolium.* The cultures are three weeks old: one-half size. (Photograph, Philip R. White.)

always roots are formed and never undifferentiated tissue. Tissue culture generally implies the growing of cells which show little or no differentiation, but this is not always necessarily the case —thus, kidney and pancreas keep to type. White, therefore, regards his plant cultures as tissue cultures just as are those of kidney and pancreas. Furthermore, although root tips never form undifferentiated tissue in culture, they also never form fully developed organs, never mature roots with secondary growth and production of xylem.

No single part or organ of higher plants or animals is ordinarily capable of autonomous existence. In plants, the root is dependent on the leaves for its carbohydrate supply, and furnishes water and salts to the stem and leaves. If part of a plant, such as a leaf or cutting of a stem, is isolated, certain cells may develop into roots and shoots. By this process of regeneration a new plant is produced. Unless regeneration of new organs occurs, the isolated part dies. The same is true of animals. The regenerative powers of some amphibian tissues are very great, yet isolated organs of these animals are incapable of long and independent existence. The severed tail of a lizard does not continue to grow as a tail. A lizard which has lost its tail grows a new one, but an isolated tail dies. This dependence of each part of the higher organism on the remainder of the body appeared to be invariable in nature. For the study of the carbohydrate metabolism of non-chlorophyllous tissue like that of the root, the experimenter has until recently been at a loss for suitable material. In animals, where isolated organs do not die immediately, it has proved practicable to keep them alive long enough to permit certain types of physiological study. The perfusion of isolated hearts and kidneys is a well-known example. The goal sought is indefinite survival in an isolated state. This has been accomplished for animal material in tissue cultures, and White has now attained it for plant material. The same clone of cultures of isolated roots of tomato is still being maintained. Complete autonomy of an isolated root has thus been reached. Isolated stem tips and cambium from trees have also been grown in culture. The evidence, now available thus indicates that, contrary to the earlier view, the dependence of plant organs on the organisms to which they are normally attached is not universal.

The nutrient used in the tomato root cultures by White is a five-salt solution similar to that of Pfeffer (page 103), but with greatly reduced phosphate content and the addition of iron, sucrose, and an extract of brewer's yeast (Fig. 70). This last can be replaced by a synthetic mixture containing certain accessory salts, vitamin B_1, and amino acids in proper proportions. In such a nutrient, and under suitable conditions, tomato roots grow in a normal manner, elongate rapidly, and branch freely.

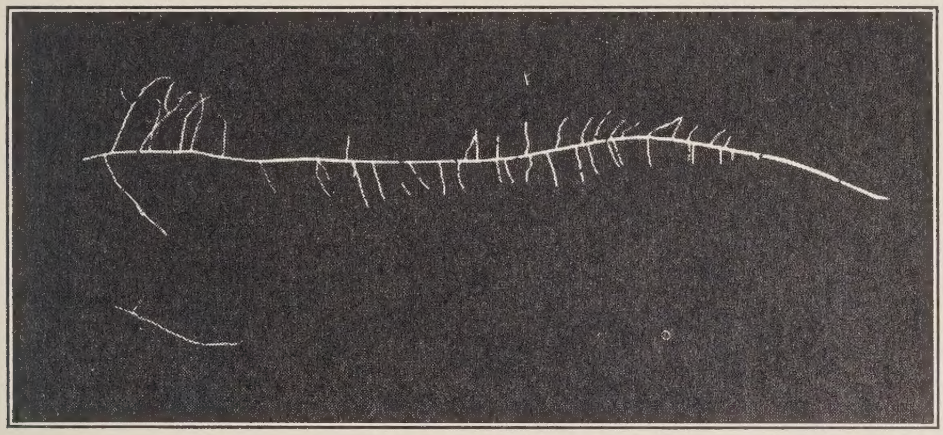

FIG. 70. Excised tomato roots, seven days' growth: top, grown in nutrient containing yeast extract; bottom, grown in nutrient lacking this accessory organic material. (Photograph, Philip R. White.)

An explant, that is to say, 10 mm of the root apex, will, in a week's time, attain a length of 125-150 mm with 20-30 branches. The roots bear a profuse growth of root hairs and possess typical root caps. The amount of differentiation attained is restricted but normal. Although all the primary vascular elements are laid down, these are not followed by development of secondary elements, cambium, etc., so that the organs remain in an immature but apparently quite normal state. They are excellently adapted for many sorts of physiological experiments in such fields as tissue respiration, temperature reactions, inorganic and organic nutrition of tissues, correlations, polarity, hormone action, infection, and tissue invasion.

From an evolutionary point of view, such cultures are of great interest, for they represent a condition which has never existed in nature, and which, if it did occur with any frequency, would greatly alter the balance as it now exists. If, when a weed is hoed up, or a potato vine pulled, or an oak tree cut down, the roots of the plant which are left in the soil should continue to grow and ramify freely without the need of leaves, there would soon be such intense competition for the available soil that plants not endowed with this ability would find existence very difficult. Isolated roots do not continue to grow because the soil cannot ordinarily supply them with the carbohydrates they require. Roots attached to green plants ramify and extend into new territory because, being supplied with carbohydrate by an agent not present in the soil, that is, the leaf of the plant, they are able to thrive in, and occupy, terrain in which organisms not so supplied would starve. It is by supplying these carbohydrates and other organic materials artificially, in culture, that roots can be made to grow as autonomous units.

PLANT CULTURE

Strange as it may seem, the possibility of extending the experimental method of water culture (page 104) to include the production of fruits and vegetables on a commercial scale was not seriously considered until 1929. Water culture as a laboratory technique was known a quarter of a century ago. It was brought to a high state of development by Gericke, Hoagland, Shive, and others. In 1929 Gericke conceived of "Aquiculture" as a means of crop production. Earlier suggestions of crop production by water culture had met with expressions of incredulity; to cover an acre of ground with quart jars, to fill these periodically with distilled water and chemicals, and to insert seedlings into corks by thousands seemed so impractical that the experimental physiologists gave the matter no further thought.

The new method of crop production by water culture has been given the name, *hydroponics,* "the art and science of crop production in liquid culture media." The term "water culture" can thus be reserved for its historic use and meaning—the growing of plants in nutrient solutions for experimental studies in plant physiological laboratories.

The method has now been developed to such a degree as to make possible the use of an ordinary grade of chemicals, such as are used for land fertilizers, and ordinary ground water. The receptacles or basins are of common building materials—concrete, wood, plastics, and sheet metal. The seedbed is a mat of vegetable litter—excelsior, straw, sawdust, peat moss, etc.—mounted over the surface of the nutrient solution. It rests on wire netting strongly secured to a portable frame, which in turn rests on the top of the basins. The seedbed provides support for the seed and growing plants, and by excluding sunlight inhibits the growth of algae. It also protects against desiccation and extreme temperature fluctuations. Hydroponics has thus become established by the use of materials and technique which heretofore have never been considered.

The success of the mass cultivation of plants by water culture depended not merely upon the mastering of physical difficulties, of equipment, but primarily upon the control of physiological limitations. Thus, roots in the soil are kept at a fairly constant temperature several inches below the surface, and this would not be true in tanks above the soil. Certain of these physiological difficulties were met by selecting suitable species. The mechanical problems proved greater than anticipated; thus, the alkalinity of cement containers had to be counteracted by leaching with water, treating with acid, and coating with non-toxic, water-resistant paints.

The elements to be added are, in the main, those found to be necessary by water culture methods (page 110). The proportions are indicated by the chemical composition of the plant.

The elements essential to plant growth can be grouped into three classes. There are, first, those required in fairly large amounts, which can be supplied in considerable excess without toxic effect; these elements are nitrogen, phosphorus, potassium, calcium, and magnesium. The second class consists primarily of the element sulphur, which can be supplied in excess without toxic effects, but is not absorbed in large amounts, hence is a minor factor in plant composition. The third class includes the elements required in small amounts which are toxic if the concentration exceeds certain low values—in many cases less than one part per million. The elements thus far recognized as belonging in this class are iron, manganese, copper, zinc, and boron; others may be added as a result of future research (page 103).

The quantities of salts chosen should conform in a general way to the proportions found in the plants. The salt which provides the cation required in largest amount should provide the anion required in largest amount. Potassium nitrate, acid calcium phosphate, and magnesium sulphate are the chief commercial sources of the major elements. As these are extensively used as land fertilizers, they are to be recommended for hydroponics.

For the growth of plants in sand and in water culture, Hoagland and Shive recommend several solutions from which the following is a selection.

SOLUTION A

MOLAR CONCENTRATIONS	C.C. PER LITER OF NUTRIENT
KH_2PO_4	1
KNO_3	5
$Ca(NO_3)_2$	5
$MgSO_4$	2
0.5 per cent iron tartrate	1

SOLUTION B

	GRAMS PER LITER
H_3BO_3	2.86
$MnCl_2 \cdot 4H_2O$	1.81
$ZnSO_4 \cdot 7H_2O$	0.22
$CuSO_4 \cdot 5H_2O$	0.08

One cc of solution B is added to every liter of solution A, which gives the following parts per million; boron 0.5, manganese 0.5, zinc 0.05, and copper 0.02. The necessity of including other trace elements in a formula for the growth of plants has not yet been demonstrated.

Twenty plants of tomatoes set in a basin 10 feet long, 2½ feet wide, and 8 inches deep—thus, containing 16 2/3 cubic feet of water, should receive nutrients in 1-pound lots, supplied as a mixture of dry salts. When the plants are about 2 months old, the nutrients are practically exhausted and then another pound is added. Subsequent additions are made at shorter intervals—7 pounds being the total amount supplied. The solution is ex-

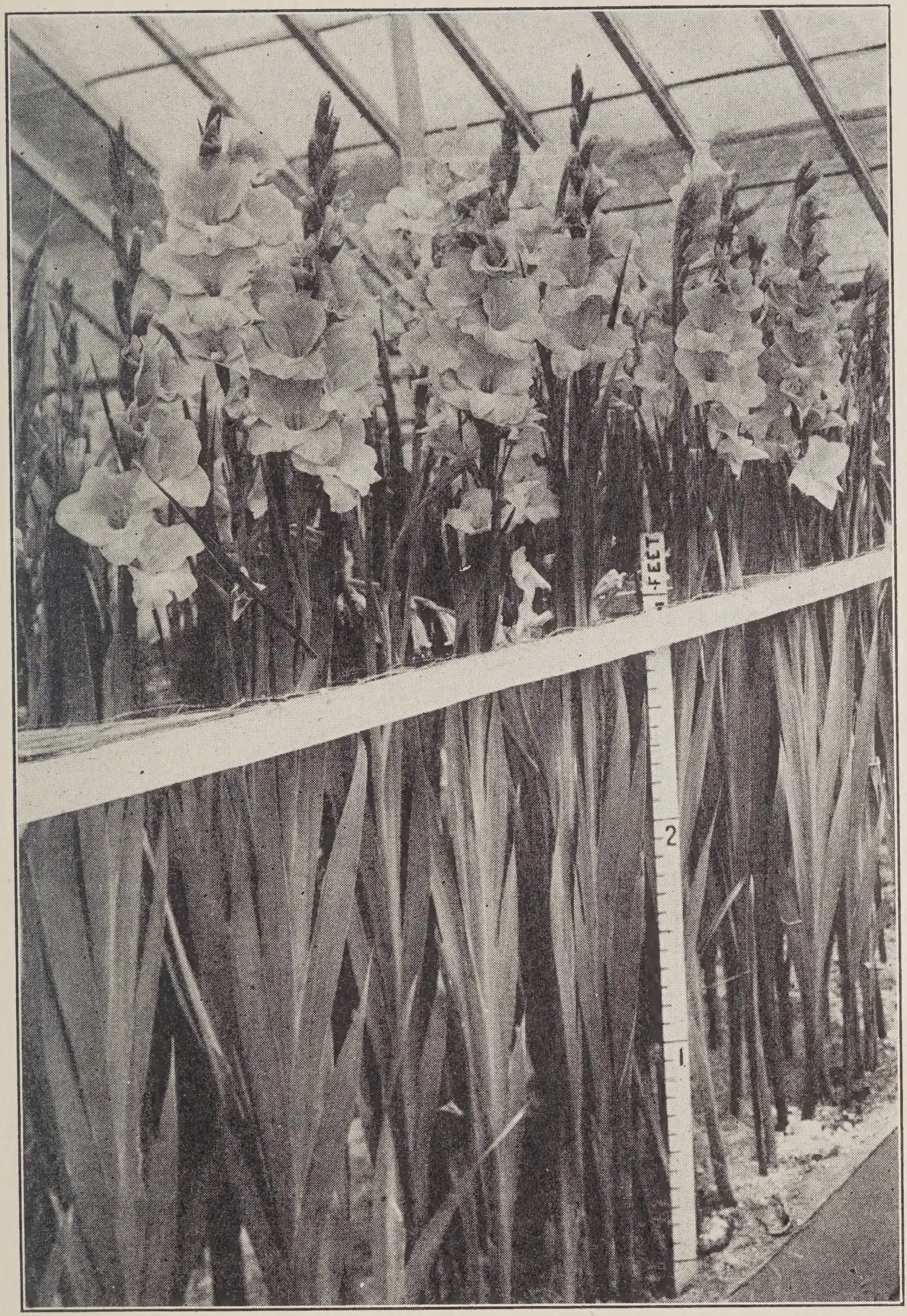

Fig. 71. Gladioli grown in culture solutions in winter. (Photograph, W. F. Gericke.)

hausted 2 months before the experiments are concluded, being then 1 year old. The average yield of tomatoes per basin is 306 pounds—more than five-sixths of the total being harvested within 9 months after planting.

The ratio of the quantity of salt nutrient required to the weight of the yield obtained makes the cost of the production of wheat and all crops that are predominantly "dry matter" prohibitive by water-culture methods under present conditions. Crops whose marketable products are characterized by high water, starch, or sugar content give promise in an economic appraisal of production by hydroponics. The cultivation of flowers by water culture should likewise prove commercially successful (Fig. 71).

These results of Gericke, Shive and others raise some startling questions. The thought that the world's needs in grass, grain, vegetables, and fruits may some day be supplied without use of soil and independent of droughts and excessive rainfall is alone sufficient to cause either great alarm or great satisfaction.

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CHAPTER XXII

REPRODUCTION

Reproduction in plants is accomplished vegetatively by adventitious buds, tubers, bulbs, runners, and cuttings; asexually by fission and spores; and sexually by the fusion of gametes. Certain of these procedures take place in the normal course of the life history of the plant, but may be affected by the environment; others are wholly dependent upon influences extraneous to the plant. It is the task of the physiologist to ascertain what these conditions are.

VEGETATIVE REPRODUCTION

Plant cuttings and the sprouting fence post are familiar examples of vegetative reproduction. Well known also is the peculiar habit of the life-plant, *Bryophyllum,* which develops adventitious buds and from them plantlets in the notches of its leaves (Fig. 57). The leaves may still be attached to the parent plant (see page 209) but are most often stimulated to bud development through severance of the leaf from the plant. *Begonia* leaves develop shoots when they are cut.

As persistent as are these examples of vegetative reproduction in higher plants, it is among lower forms that this property is most highly developed.

As the main shoot of mosses advances, the posterior portion dies, forming isolated lateral branches each of which becomes a separate plant. The moss protonema may arise not only in the usual way from spores but also from any part of the plant, from stem, leaves, rhizoids, or sporangium. The development of the protonema from spores is a normal heritable process functioning more or less independently of the environment, but accessory protonema development is influenced, if not determined, by the environment, light and moisture being important factors.

Vegetative reproduction sometimes supplants normal sexual reproduction owing to physiological disturbances. In certain ferns, e.g., *Pteris cretica,* a young plant may arise from a bud formed on the prothallus. This represents asexual reproduction by the gametophyte.

ASEXUAL REPRODUCTION

Among the lowest forms of life, *fission* is the usual form of reproduction. The time and rate of fission of one-celled plants are to a great extent determined by environmental conditions.

Spore production is a common form of asexual reproduction among algae, fungi, mosses, and ferns. Spores from the same plant may appear to be identical and yet possess significant physiological differences. That the spores are different is shown by their behavior. Some of the isospores of a dioecious liverwort such as *Marchantia* give rise to male plants and some to female plants. That these spores are sexually distinct is not externally evident. The difference lies in the nature of the protoplasm.

The alga, *Ulothrix,* produces three types of swarm spores, macrospores, asexual microspores, and sexual "microspores"; the last fuse and are, therefore, not true spores but gametes. These sexual "microspores" differ but slightly in appearance from the asexual ones, yet physiologically they are quite distinct. On the other hand, microspores and macrospores are outwardly readily distinguishable but differ little physiologically.

Fusion of the sexual "microspores" or isogametes of certain species of *Ulothrix* takes place normally only between gametes from distinct threads. The gametes cannot be termed male and female, for they are alike in appearance and so they are said to be of distinct strains.

SEXUAL REPRODUCTION

Eggs are fertilized by the sperm of the same species, but not usually by the sperm of another species. Rarely the egg and sperm of the same species will not fuse. Biological or chemical differences between sperm and egg, between individuals, and between species are responsible. Why the sperm and egg of different species, and even sometimes of the same species, will not fuse, it is difficult to say; physiological factors, such as protein incompatibility, or genetical factors, such as lethal genes, may be postulated. Before attempting an answer to these questions, it is first necessary to explain why gametes fuse at all. The benefit of fusion is evident; there is a mixture of strains. The act of fusion must be preceded by some directive substance or force. The sperm is guided to the egg, and the pollen tube is guided to the embryo sac. The solution of this problem is best sought in lower organisms.

The sexual reproduction of the green alga, *Spirogyra,* and of the bread mold, *Rhizopus,* is primitive. Filaments from plants of opposite strains form tubes which come into contact, and fuse. The conjugating filaments are similar in appearance, but they will not join with any chance filament; the mate must be of an opposite strain. If the filament of an appropriate strain lies close by, conjugation ensues. It would seem that there is some means of communication between the two plants, for the direction of growth of the tubes is never haphazard, but always determinate, one toward the other. It is unlikely that one explanation will fit all cases, but it seems very probable that in most instances where the fusion of gametes occurs, a substance is secreted by the female, either the gamete, or its receptacle; for this substance the male gamete is positively chemotactic. The presence of such a substance has been demonstrated in some plants.

Free-swimming isogametes secrete a specific sexual substance which induces fusion, and, when this substance is not produced, no union takes place. Moewus has proved this by inducing conjugation between gametes of *Chlamydomonas* in the dark. The specific substance necessary for fusion is not produced in darkness, but, if the filtrate from gametes exposed to light is added to water containing gametes in the dark, they conjugate. It thus appears, as Fritsch says, that "there exists in every gamete a hunger for some substance which only the opposite strain or sex can produce."

In free-floating eggs, such as those of *Fucus*, which are fertilized in the sea after discharge from the parent plant, a secretion attractive to the sperm is formed at the egg surface, as shown by the behavior of the sperm following fertilization. A fertilized egg develops a fertilization membrane immediately after a sperm has entered. Were the fertilized egg chemically and physiologically the same as the unfertilized egg, then sperm would continue to attempt to enter, but they do not. They are prevented from entering, not only because of the mechanical obstacle set up by the fertilization membrane but also because no substance attractive to them is secreted. The failure of sperm to attack a fertilized egg, in contrast to their energetic assault on an unfertilized egg, is evidence of the presence of an attractive substance surrounding the latter.

SEXUAL RHYTHM

The sexual cycle in many plants and animals proceeds with great regularity, as illustrated by those extraordinary cases of life cycles in bamboos and palms which are so precise that they are repeated generation after generation with not more than a six months' deviation out of thirty-two years (page 231).

All behavior phenomena may be said to be determined in part by heredity and in part by environment. To what extent each is responsible is an open question; it may be a matter mostly of heredity in one case and of environment in another. Certain investigators, such as Klebs, regarded environment as the chief determiner of a plant's activities. Others attribute the behavior of plants and animals primarily to the innate heritable qualities of their protoplasm.

Sexual rhythm is a heritable quality and is often undisturbed by change in environment, but it may in cases be guided by the environment. Thus, the seasonal rhythm of plants is a heritable quality, but not without influence by the environment which determines when each stage in the cycle is to begin and end.

More firmly established, and therefore more independent of the environment, is the sexual rhythm of the brown alga, *Dictyota.* Hoyt found the sexual rhythm in this organism to be so deep seated that plants kept in the laboratory for several weeks discharged eggs and sperm at precisely the same time as did the plants in the sea, and continued to behave in this manner even when the fruiting branches were developed in the laboratory. The advantage of the simultaneous and gregarious discharge of eggs and sperm lies in the fact that fertilization is more certain if large numbers of both are discharged within a single hour instead of being liberated throughout every hour of every day. In striking contrast is the behavior of the asexual plants growing in the same locality; these discharge their tetraspores at any time and not simultaneously.

ENVIRONMENTAL INFLUENCES

Zoospore formation in some filamentous green algae may be induced by so simple a change in environment as transference from flowing water to still water. Change in illumination and transference from soil to water accomplish the same result in other forms of algae. Light inhibits and darkness induces zoospore formation. There seems also to be some evidence that alkalinity induces microzoospore formation in *Draparnaldia.*

It is a general rule in plant life that favorable conditions tend toward purely vegetative growth whereas unfavorable conditions tend to bring on reproduction. This rule is brought into practice by European horticulturists when they weaken a plant by trimming the roots and thus bring about better fruiting in trees which have shown great vitality but no reproduction.

There is some evidence to support the general rule that unfavorable conditions favor reproduction, but in most plants reproduction usually follows a period of great metabolic activity, culminating in the accumulation of much food; in fact, reproduction is said to be induced by excessive storage of carbohydrates. Such conditions can hardly be called unfavorable from the nutritional point of view. That sexual activity begins at the completion or climax of vegetative activity when the food supply is at a maximum is not proof that carbohydrates or other stored food are the cause of reproduction. The two conditions are merely concomitant—but it is certainly proof that starvation is not the usual cause of reproduction in plants, though it might at times be an inducing factor.

Drought is thought to hasten and possibly cause the reproduction of plants, in particular bamboos, which exhibit such remarkable gregarious flowering (page 232). Closer investigation, however, shows that neither drought nor heavy rains affects the time of flowering. The sexual rhythm functions independent of the environment.

Garner and Allard in their work on the influence of light on the time of flowering of plants were able to cause fall-flowering asters to blossom in the spring, and to postpone the flowering of annuals until the following year (Fig. 89). These changes in the sexual rhythm of plants were accomplished by changes in length of exposure to light (page 288). Short days hasten reproduction—too little light is an unfavorable condition; long days delay reproduction—abundant light is favorable to vegetative growth.

The results of Garner and Allard are fundamental, but it must not be assumed that more than a hastening or retarding of the time of flowering has been accomplished; the annual does not become a biennial merely because it lives in two calendar years. A biennial grows vegetatively the first year, and the second year forms a new and different type of stem, which reproduces; this has not been accomplished by merely postponing the flowering of an annual.

VERNALIZATION

When man desires to adjust the development of plants to his will for practical purposes, or sometimes merely to see what can be done in the way of changing the course of nature, he often obtains unexpected results which seem to indicate that natural development is not always as rigid as usually assumed. Attempts to change the course of development in plants by accelerating reproduction through practical agricultural methods are known as *vernalization*, which means acquiring the properties of early flowering or of spring plants. The word is a latinized form of a Russian word of similar meaning.

The original method of vernalization involved soaking seeds so as to transform them from a state of rest into one of active growth, and then, as soon as the embryo had begun to emerge through the seed coat, applying a stimulus, such as low temperature, which later accelerates development. If "vernalization" includes all means for hastening the development of plants, then other methods of forcing must be included, such as shortening the time of exposure to daylight (page 288). Lysenko has been able to convert winter annuals, plants sown in the fall for spring flowering, into plants which may be sown in the spring and yet will flower the same summer. He accomplished this by soaking the seeds of winter plants, e.g., winter wheat, and then, while germination is going on, subjecting them to a temperature slightly above freezing over a period of fifteen to sixty days. The seeds are sown in the spring, and they yield fruit the same summer, frequently earlier than spring varieties of the same species. The factor accelerating development in this case is low temperature, but some plants respond to relatively high temperatures. Such so-called *thermophilic* plants include millet, soy bean, and cotton. When the germinating seeds of these plants are exposed for several days to a temperature of 20° to 30°C, they attain maturity in a cool climate, e.g., in North Russia, where fruiting would otherwise be impossible.

HYBRIDIZATION

Cross-pollination occurs readily between individuals of the same species; in fact, nature has contrived to assure it by very special means in many instances. Cross-pollination between different species of the same genus is frequent but not general. Cross-pollination between genera is very rare, and most of the few instances of it on record are open to question. Crossing between members of different families is unknown. From a genetical standpoint, there is good reason why the pollen of one species should not fertilize the egg of another species, for in nature the line is usually kept pure. Such a reason will not satisfy the physiologist. There must be a physiological cause for the failure of one species to fertilize another. Incompatibility between the proteins of the two species is a possible interpretation, or it may simply be the lack of an adequate activator to start growth or improper nutrition for continued growth of the pollen grain. Attempts to germinate certain pollen grains in artificial culture media proved unsuccessful until a chemical analysis of the sticky substance on the stigma of the species revealed the presence of boron (page 110). When this element was added to the medium, germination took place immediately. The same situation may exist in cross-pollination, one species lacking the catalyst necessary to cause the pollen of another species to grow. Probably not all unsuccessful attempts at cross-pollination rest upon quite so simple a maladjustment. Germination of the pollen grain may take place, but, owing to incompatibility between distantly related species, fusion of the male and female nuclei does not follow.

The most satisfactory interpretation of sexual incompatibility is the phenomenon known as *allergy*, or the incompatibility of proteins. It is best illustrated in man; for all individuals are sensitive to some particular protein. Hayfever, strawberry rash, and forms of eczema are manifestations of incompatibility between the proteins of the human body and the proteins of plants. Similar allergic reactions may be shown by plants to the proteins of other plant species.

Bigeneric crosses are very rare and always take place between closely related genera. The physiologist may, therefore, say to the systematist that if crossing is possible then the species are of the same genus; in other words, the greater the incompatibility between proteins, the more distant is the relationship. Successful cross-pollination is evidence of protein relationship and therefore of generic relationship. The work of Mez indicates that this is true (page 184). The cross between Mexican teosinte and Hopi maize would thus have to be regarded as intergeneric and not bigeneric, as the geneticist now regards it. Such questions emphasize the need of a physiological basis of plant classification, or a physiological verification of the validity of purely morphological criteria of relationships.

HEREDITY

The physiology of heredity involves a consideration of the qualities of protoplasm and the nature of its environment. Darwin thought that variations were caused by changed environmental conditions and that, "were it possible to expose all the individuals of a species during many generations to absolutely uniform conditions of life, there would be no variability." Exception must be taken to this view, for it seems that, although protoplasm cannot be wholly dissociated from its surroundings, it may yet undergo permanent change in a stable environment. For example, apparently identical sex cells, produced under like conditions and existing in the same environment, may behave quite differently. Thus, it sometimes happens that the gametes of *Ulothrix*, normally destined to fuse, fail to do so and germinate independently. Such behavior would appear to rest upon internal and not external conditions.

The foregoing behavior of the gametes of *Ulothrix* is an example of *parthenogenesis* or development without fertilization. Not only the one gamete, which may be regarded as the egg, develops parthenogenetically, but the other gamete, which may be regarded as the male, also does so. Male parthenogenesis is not known among higher plants, but, as Vines points out, this may be true simply because the male gamete is greatly reduced in cytoplasm and lacks the nutritive qualities for independent germination.

The inheritance of acquired characters was rather generally accepted as true at the time that Darwin maintained that sudden variations are not permanent whereas those slowly produced through the accumulative action of changed conditions of life are. At the present time this hypothesis is in disfavor; sudden changes or *mutations* are regarded as the chief contributors to organic evolution. In spite of present-day opposition to the Darwinian hypothesis, it seems rather improbable that environment, which can do so much in the way of changing the path of autogenetic, or individual, development (pages 105, 284), should be wholly ineffective in directing phylogenetic, or race, development. The concept of adaptation is meaningless if the effect of environment is denied. The acceptance of adaptation as a principle does not necessarily involve teleological, purposeful, or directive implications; it simply implies that plants possess mechanisms which make it possible for them to live under the conditions of their surroundings. Whatever the point of view, it must be admitted that a cactus is well adapted to survive on desert soil, whereas a tropical rain-forest plant would not survive twenty-four hours. If the modifications in plants which fit them for their surroundings have come fortuitously and therefore are not expressions of environmental influence, then at least environment has been instrumental in selecting from among the chance variations those that are adaptive and will survive.

PRACTICAL APPLICATIONS

The practical applications of physiological problems in reproduction are many. The practice of horticulturists in trimming roots to induce fruiting has been cited. French gardeners grow their plants under intensive conditions. Fruit trees in America are allowed to spread and grow much as they wish; but in France they are often trained flat against walls to conserve space and gain light and warmth. Pruning is most carefully done; it is a daily or weekly, and not just an annual affair. Among the rules is one requiring that at least two leaves be left beyond the fruit on the twig which bears it. This is done to insure nourishment for the fruit. The leaves "draw" water and salts, and send carbon compounds in return. This food supply nourishes the apple.

Tropical horticulturists have their problems too. Among the most uncertain of crops is cacao. The cacao planter not only has fluctuating prices and the witchbroom parasite to worry him, but also the "setting" of his fruit. The number of young fruits is no indication of the final harvest, for until the pods are "set," any disturbance, such as drought, may cause them to fall. Setting is due to a very fundamental event within the seed. The fertilized egg in a cacao ovule does not segment until fifty days after pollination. During this quiescent period the embryo, which is still but a fertilized egg, makes no nutritional demands on the tree, and the pod is, therefore, not firmly attached. Once development of the embryo gets under way, there is a "pull" on the food supply which establishes a new relationship between seed and parent. The fruit is then "set," attachment is secure, and subsequent loss unlikely.

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CHAPTER XXIII

PLANT MOVEMENTS

MOVEMENTS IN GENERAL

Movement in plants may involve the plant as a whole or only parts of it. These parts may be larger ones such as the root, stem, and leaf, or smaller ones such as cell inclusions, protoplasm, and the body fluids. The parts may be detached as are seeds, pollen, and gametes. The movement is in most cases an autonomous movement, but may be wholly passive, as in the dispersal of seeds. Attempts have been made to classify plant movements but not usually with much clarity. An expression such as "paratonic movements of variation" is of little help to any one except a specialist in the subject. A classification may be based on the mechanisms involved, whether growth, as in the case of tropisms, or other causes. But this, again, is of little help because in many instances the responsible mechanism is not known, and until this is known it is best to tell simply what happens, and then suggest the ways in which the movement might come about. Classification may also be based on the responsible stimuli, such as light, gravity, chemicals, and shock.

PROTOPLASMIC MOVEMENTS

Protoplasm is never wholly and permanently quiet, for living matter is dynamic, not static. In some cells, such as slime molds, the protoplasm is in a constant state of motion; in other cells, such as those of *Elodea*, the protoplasm is at times quiet and at other times very active. The mechanism of protoplasmic movement has long been the subject of speculation among physiologists. The physical forces and chemical reactions which have been resorted to in an attempt to find the source of energy responsible for protoplasmic flow include surface tension, hydration, electroendosmosis, cataphoresis, oxidation-reduction potential, and rhythmic contraction. The mechanism of protoplasmic streaming has been given consideration elsewhere (page 43).

MOVEMENTS OF CELL PARTS

In addition to protoplasm as a whole, certain cell parts carry on movements which may be passive or active. *Mitochondria* are carried about in a cell by the protoplasm in which they are suspended. The hypothesis has been advanced that mitochondria are independent organisms, and their movement, therefore, autonomous, but there is no real evidence to support this. The independent trembling motion of mitochondria is of another kind, namely, Brownian movement (page 18).

Chloroplasts, too, move passively, carried by streaming protoplasm, but they also move independently in response to intensity of illumination: this is known as *phototaxis* (Fig. 72). How the chloroplast accomplishes its phototactic movement is unknown. There is no evident motor organ. The position of the chloroplast, with its broad face next to the wall, is the normal one for a body so shaped to take in a current, but other positions assumed with change in illumination oppose the protoplasmic current. The chloroplast adjusts its position so that its broad surface is turned toward weak light and its edge toward strong light.

Chromosomes migrate to their respective poles in a dividing cell. Their movement has been attributed to magnetic forces; to contraction of the spindle fibers, and to ameboid movement. The magnetic hypothesis is excluded by an experiment showing that a powerful electromagnetic field has no effect on the mitotic figure of a dividing egg.

Cellular movement, that is to say, the motion of free, unicellular organisms is brought about in a number of ways. The ameboid movement of slime molds and amebae is accomplished by the extrusion of pseudopods which adhere to the surface of the substratum while the rest of the protoplasm flows forward. The physical basis of pseudopod formation has been said to be due to a reduction in surface tension at the point where advancement takes place. Surface tension changes are undoubtedly involved, but whether as cause or result of the movement is not known. Plasmodia and amebae do not appear to be wholly amenable to surface tension laws. Surface forces come into play in active protoplasm, but they can be opposed or modified by internal forces.

The swaying or oscillating movement of the alga, *Oscillatoria,* is an interesting and wholly unexplained form of motion. The filaments of this blue-green alga sway back and forth with no obvious means to accomplish it. Protoplasmic protrusions, and an alternate shortening and lengthening of the opposite sides of the filament wall, are suggestions of a possible mechanism.

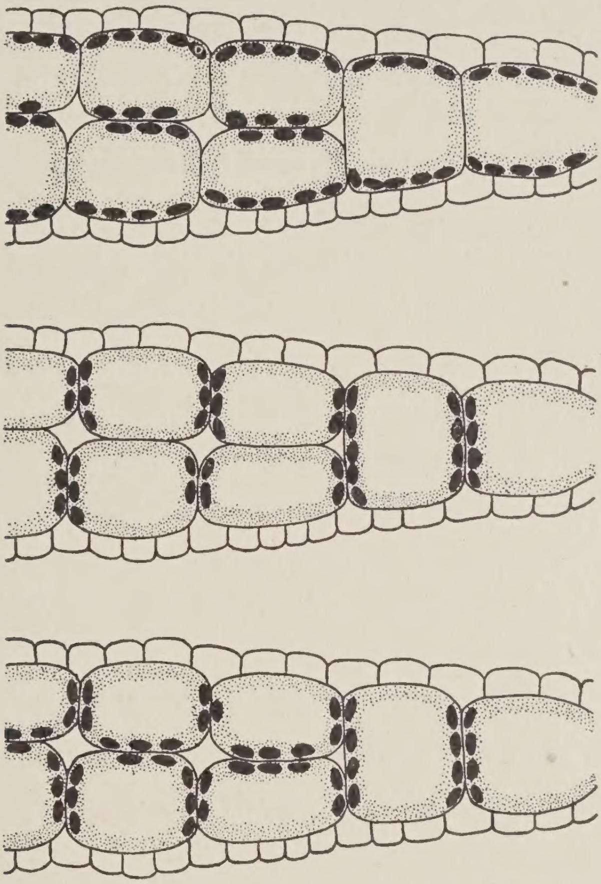

FIG. 72. Phototaxis, illustrated by the orientation of chloroplasts with change in direction of light: top, medium light; center, strong light; bottom, weak light. (Redrawn from Pfeffer.)

Diatoms move or glide along, presumably by protoplasmic bands which leave the silicious shell at one point, pass along a groove, and enter at another point. Movement of this kind is somewhat like that of a caterpillar tractor.

Bacteria, flagellates, motile spores, and gametes move by means of flagellae, and cilia, or whip-like processes. *Bacillus subtilis* is a motile bacterium with eight to twelve cilia, and *Euglena viridis* a flagellate with a single terminal flagellum, whereas gametes and zoospores are propelled by one or many flagellae. Cycads and gingkoes are the highest plants in the vegetable kingdom to have motile male sex cells; these spermatozoids are wrapped with a fringe of many cilia resembling the string on a top. Whipping the water with flagellae is the method of locomotion for many free-swimming cells.

MOVEMENTS OF HIGHER PLANTS

Plants, other than unicellular, free-floating, or swimming ones, are distinguished from animals by the fact that they do not move from the spot where they first take root. Certain plants, however, move in so far as they spread by special means. The "walking-fern," *Camptosorus,* and several other fern species form buds at the tips of their slender fronds which take root when the tip of the frond droops and touches the soil. The second plant thus produced may repeat this movement and so form three plants in a row. The banyan tree (*Ficus* sps.) spreads by forming adventitious roots which hang from the long, horizontal limbs and grow until they reach the ground, enter the soil, and thicken, thus forming secondary "trunks" which morphologically, remain absorbing roots. A banyan may cover an acre of ground. Such movements of the plant as a whole are not of great concern here, for, though of interest, they do not appear to rest upon any particular physiological condition but are due rather to heritable qualities of the species.

THE DISSEMINATION OF SEEDS

Seed dispersal is, essentially, a morphological or structural problem but the relationship between the anatomy of the seed and its environment brings seed dissemination into the realm of physiology. Many seeds have special means to aid their dispersal. Some birds when they eat fruits swallow the seeds which pass through undigested. Seeds, such as burrs, cling to animals and are thus disseminated. A seed with wings, like the maple key, is carried by the wind. Seeds and fruits with floating devices, such as air sacs or a fibrous husk, as in the case of the coconut, are borne upon water and often float great distances. Linnaeus recorded the presence of seeds and fruits from tropical America on the coast of Norway, whither they had been carried by the Gulf Stream.

HYGROSCOPIC MOVEMENTS

Plants sometimes accomplish the dispersal of their seeds and spores by the movements of structures due to wetting and drying. Such *hygroscopic movement* is accomplished through the repeated hydration and dehydration of cell walls. It is illustrated by the coiling and uncoiling of the spirally formed elaters in the capsules of liverworts and by the peristome teeth of mosses which, moving hygroscopically, disperse the spores. Fern sporangia are torn open by the annulus owing to its curvature when the thin outer walls collapse on dehydration of the cells, the inner heavy walls remaining relatively unaffected. A spiral twist often results in plant structures when they dry. Certain pods are torn open on drying owing to the unequal contraction of the two sides of the wall; the inner surface of the wall contracts less than the outer surface; this results in the twisting of each half of the pod and its rupture.

MOVEMENTS ACCOMPLISHED BY TURGOR

Living plant cells are turgid owing to the osmotic pressure of the contained solutions. Increase in turgor or a lessening of it will, especially when sudden, bring about a movement of the structures involved. The ejection of spores from the sacs or asci of the fungus, *Ascobolus,* is accomplished by turgor. As the asci ripen, the turgor of the cell increases until, at maturity, the delicate membrane at the tip of each sac can no longer withstand the pressure, with the result that the ascus is suddenly ruptured and the spores ejected with considerable force.

The turgor of the cell or the imbibition pressure of the mucilaginous contents of the fruit of the so-called squirting cucumber brings about the forceful ejection of the seed when ripe.

The stamens of certain flowers (*Spartium*) are suddenly released when visited by insects owing to the turgor of the cells. The release of the pollen sacs of those orchids which are so nicely adapted to pollination by the bee and the later bending of the stalks which are attached by cementing discs to the head of the insect are accomplished by osmotic or imbibition forces. The ejection of the pollen of certain Urticaceae, as well as members of the genus *Kalmia*, is due to the sudden release of the stamens which are held in a bent position under tension while the flower is opening; on maturity the bent position is overcome by turgor.

The bending of the tentacles of the sundew, *Drosera,* and the closing of the halves of the leaves of the Venus flytrap, *Dionaea,* are movements due to induced changes in turgor (Fig. 84). Still another example of this type of mechanism is furnished by the sensitive plant, Mimosa (see pages 48, 273).

GROWTH MOVEMENTS

When growth consists merely in increase in size, or distention, it is most often due to the turgor or osmotic pressure of contained fluids, but plants exhibit other forms of growth movements. Plants with a central stem describe a circular or, more accurately, a spiral movement as they advance upward. The motion is very slow, but by marking the position of the tip of the plant at any one time and observing it some hours later the change can be seen. There is no evident benefit in this motion, as in other spiral movements such as those of tendrils and vines. Spirality in growth is a common characteristic of organisms (page 233).

When tendrils come into contact with a support the repeated irritation on one side either inhibits growth there or accelerates it on the opposite side. Resort to a growth-inhibiting or a growth-stimulating hormone is in keeping with the present-day work on hormones but the postulates necessary complicate the situation. Irritation of the tissue on the inner side of the tendril might arouse the cells there to hormone production, but the assumption would have to be made that the substance produced was either a growth inhibitor or if growth-promoting it would have to migrate to the outer side of the tendril stimulating the cells there to greater activity. In both cases curvature would result.

Tendrils are capable of making either right-hand or left-hand twists, but most vines appear to be definitely inclined to twist either to the right or to the left (Fig. 66). A right or left spiral path of terminal growing points should be investigated.

The cause of the rotatory movements of growing plants, whether in straight stems or in twining vines, is not understood. The motion does not take place if the plant is kept revolving. Some investigators are of the opinion that the movement is the direct result of geotropic influence, or gravity. Other investigators regard it as an expression of inner forces; possibly both inherent and environmental influences combine to produce the motion.

HYDROTROPISM

Internal factors are responsible for growth, but the direction which the growing parts take is determined by external influences. Among these is moisture. A movement or tropism directed by moisture is known as *hydrotropism.* Roots are very commonly positively hydrotropic. It is quite obviously a benefit to the plant if its roots have the natural tendency to bend from a region of low moisture content to one of high moisture content.

CHEMOTROPISM

A growth curvature which is determined by a chemical reagent is known as *chemotropism.* There are numerous examples of chemotropic responses, among them being the path followed by sperm in their journeys to eggs, a phenomenon which defies interpretation on any other ground except chemotropic response: the sperm, once having entered the farthermost edge of the chemical substance, proceeds from a region of low concentration to one of high concentration (page 251). The even more baffling problem of the path taken by the tubes of pollen grains in their journey through the pistil to the egg is probably another case of chemotropism.

Roots illustrate chemotropism; positively so when they are attracted by a suitable concentration of salts; negatively so when they retreat from toxic substances.

GEOTROPISM

As gravity is an invariable influence, plants are guided by it in their growth. Stems grow upward and roots downward because of the effect of gravity. If a plant is put upon its side, or held upside down, in a short time the stem will turn upward (Fig. 73) and the root will grow downward, bending near the tip. These changes in direction of growth take place whether the plant is in the light or dark, whether moist or dry, as long as the position remains out of the vertical.

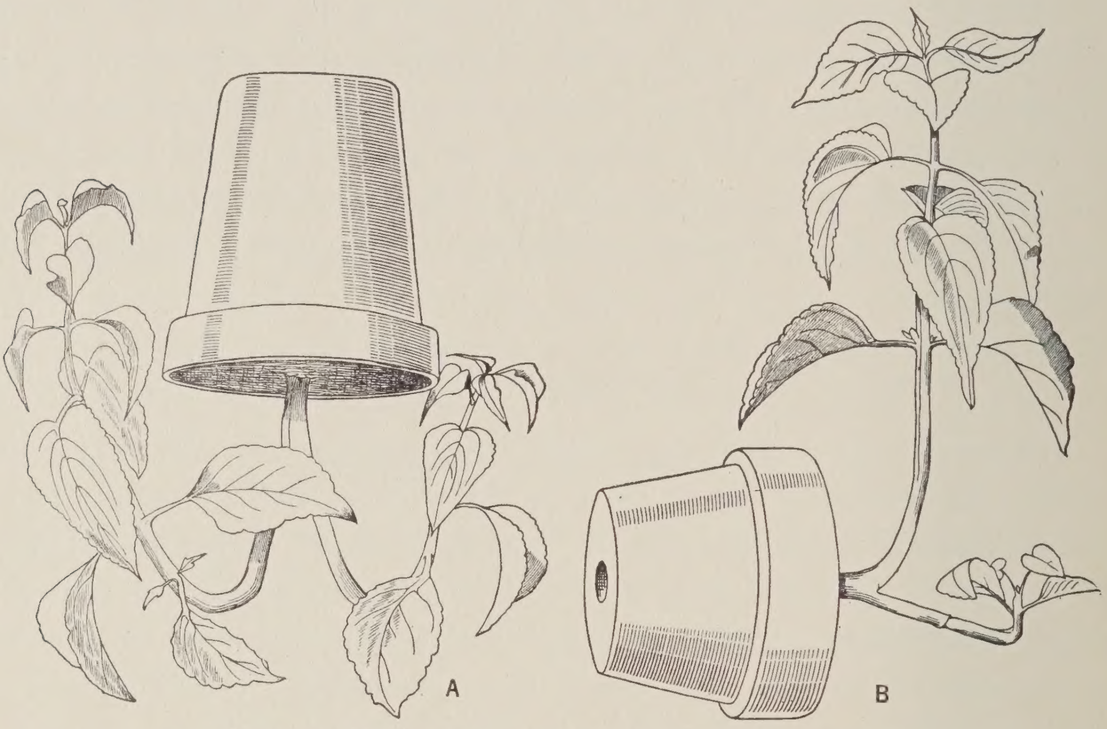

FIG. 73. Negative geotropic response to stems.

Stems which grow against the force of gravity are said to be *negatively geotropic*, and roots which grow in the direction of gravity are said to be *positively geotropic*. Lateral stems differ in their response to gravity; in some trees the branches are *plagiotropic*, i.e., neutrally geotropic, and grow horizontally; in others they point at a 45° angle, and in still others, such as the Lombardy poplar, the lateral branches grow almost vertically.

The effect of gravity on the stems and roots of plants is evi-

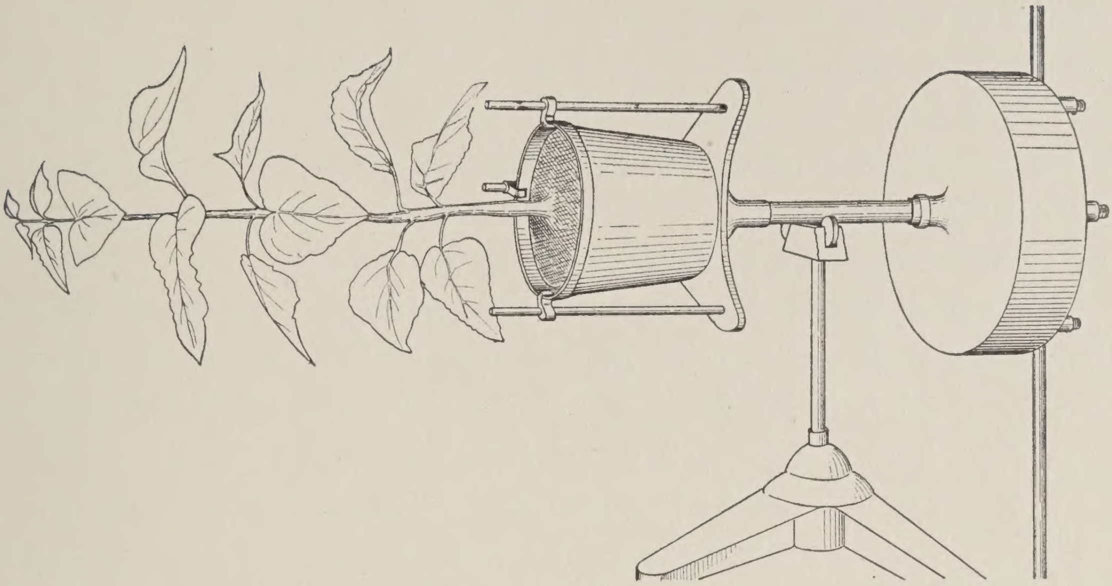

FIG. 74. Klinostat with potted plant, demonstrating absence of geotropic response of stem when plant is rotated.

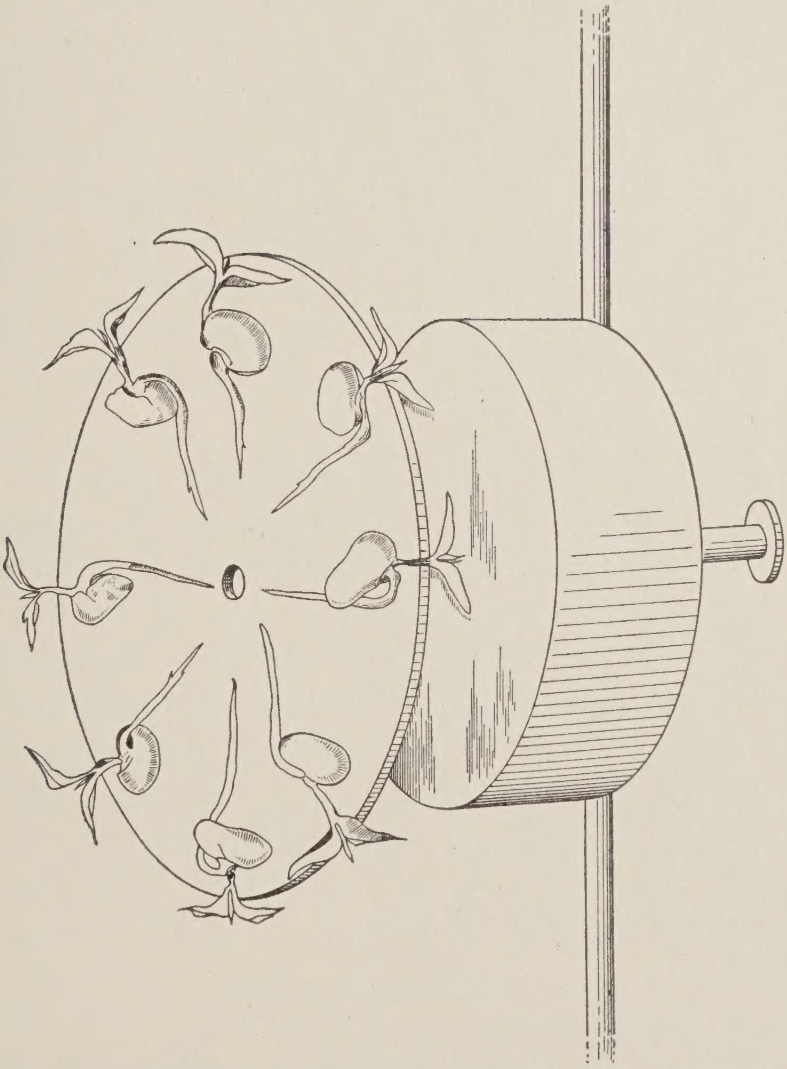

FIG. 75. Klinostat with seedlings, demonstrating absence of geotropic response in roots when seedlings are rotated.

dent the moment these organs protrude from the seed; the plumule immediately turns upward and the radicle downward. If the seed has been placed "upside down" in the soil, the young stem makes an "about face" even though in so doing it may have had to turn through 180°, and the young root makes an equally great bend in order to grow down into the soil.

If gravity is responsible for the upward growth of stems and the downward growth of roots, then neither the stem nor the root of a germinating seed should show negative or positive geotropic growth if attached to a vertical rotating disc. This is the case; the plant stem and root continue to grow in the direction established before they were attached to the *klinostat* (Figs. 74, 75).

Geotropic curvature can take place only in those regions of a stem or root where the tissue is still in a formative stage. Tissue which is well advanced in its development is no longer capable of responding to gravity either because the cellulose is not sufficiently plastic to yield, or because the cells, being mature, do not respond. Curvature, therefore, takes place only in the meristem of roots and stems where cells are still young (Fig. 76).

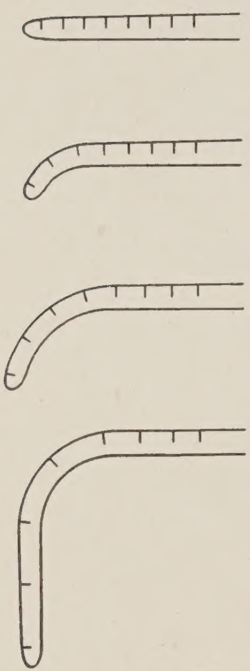

Fig. 76. Seedling roots, illustrating that curvature in response to gravity takes place only in the region of active growth.

The cause of geotropic response, that is to say, the internal mechanism, is unknown. Several suggestions have been made. Haberlandt thought that the starch grains lying at the bottom of cells may possibly serve as gravitational sense organs; he called them *statoliths* (Fig. 46). If a stem is turned upside down, the statoliths slowly fall until they again rest at the bottom of the cell. As the statoliths do not normally rest at this end of the cell, the stimulus presumably serves to bring about a response.

PHOTOTROPISM

Geotropism is the first stimulus to which the young plant reacts, and although it is always the predominating influence, it is often modified by *phototropism* or *heliotropism.* Phototropic movements are due to the effects of light. The turning of leaves toward the light is a very familiar example of phototropic reaction (Fig. 77), so also the daily motion of the sunflower and the leaves of many trees as they follow the sun with considerable precision from sunrise to sunset. The position assumed by the leaves of the compass plant, *Silphium laciniatum,* is another striking example of phototropic response, for, when viewed from the north or south, only the edges of the leaves are seen, but from the east or west the broad surfaces of the large blades present themselves.

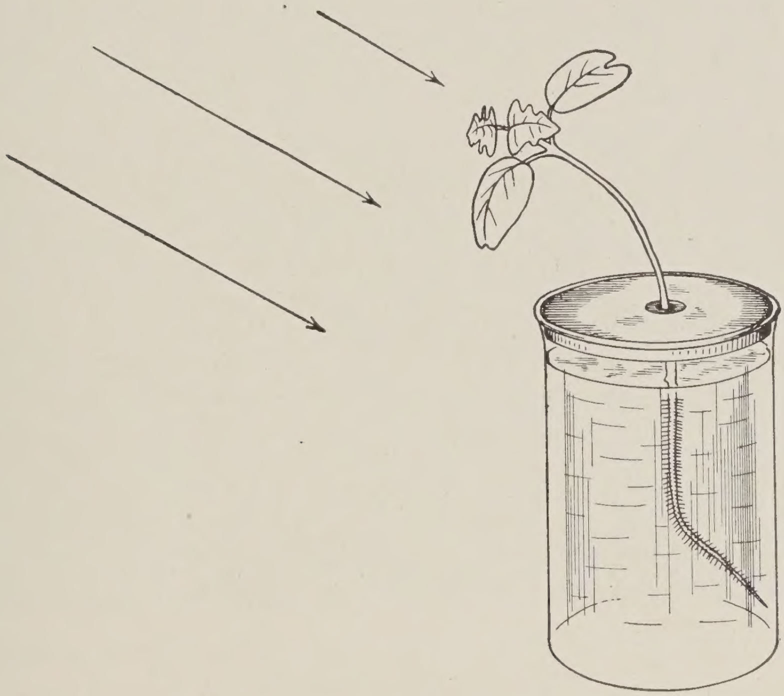

Fig. 77. The positive phototropic response of a stem and negative phototropic response of a root. Light enters at the left.

Where leaves assume a permanent position, as in the compass plant, there can be no question but that this change is gradually acquired through growth, through greater cell division on one side than on the other; a twist without torsion results. But where the phototropic response is a daily one, torsion, due to osmotic forces, may be the mechanism of the movement, and not growth. However, it is generally assumed that all tropisms are growth reactions.

Uneven growth is a very simple explanation of phototropic response. Positive phototropism in stems results because light retards growth; consequently, that side of the stem which is toward the light will grow less than that away from the light, and the stem will bend toward the light. But what of negative phototropism in roots? Here light should accelerate growth so as to produce curvature away from it. The hypothesis requires that stem tissue shall show one kind of response to light and root tissue the opposite kind. That this is true is evident from the fact that stems and roots do respond differently to both light and gravity; where the one is positive the other is negative.

Some tissues vary in their response to light according to conditions, such as the intensity of the illumination. Certain algae are positively phototropic in weak light and negatively phototropic in strong light; in general, the intensity of light affects plants more than the quantity. The minimum light intensity to which plants will respond varies with species.

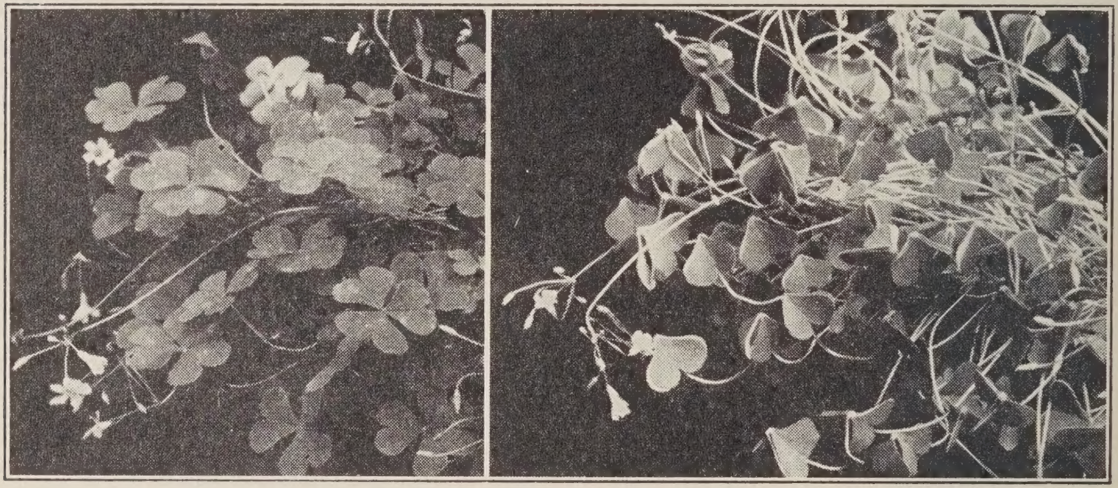

FIG. 78. *Oxalis:* left, leaves open in daytime; right, closed at night. (Photograph, Walter Steckbeck.)

The physiological or ecological value of phototropic movements to higher plants comes from better illumination of the leaves and therefore greater photosynthetic activity.

Stems are negatively geotropic and roots positively so, and stems grow into the light and roots into the darkness. The question arises, which influence is the stronger? Although stems and roots react primarily to gravity, light will influence their growth sufficiently to divert them from the straight axis which they strive to maintain under the influence of gravity; thus phototropism may, within limits, prevent geotropism from exercising its full influence (Fig. 77).

Motile unicellular plants and motile spores respond to light. Zoospores generally move toward light of moderate intensity and are repelled by very strong light. The microzoospores of *Ulothrix* show greater sensitiveness to light than do macrozoospores of the same plant.

Phototropic and geotropic responses are characterized by two qualities, the *latent period* and the *after effect.* The time elapsing between the reception of a stimulus and the subsequent response is the latent period. Pfeffer found that the phototropic response of pulvini (Fig. 80) commences one minute after illumination. Geotropic reaction may require twenty to thirty minutes.

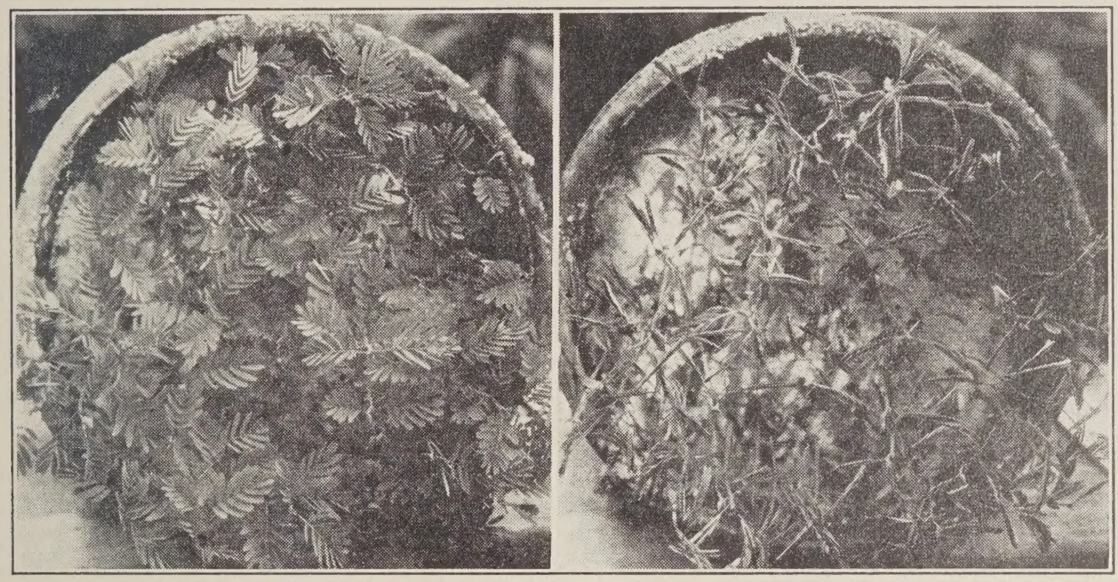

FIG. 79. *Cassia nictitans:* left, leaves open in daytime; right, closed at night. (Photograph, Walter Steckbeck.)

Response to a stimulus may take place after the stimulus is removed, that is to say, there may be quite a prolonged maintenance of the response after the stimulation has ceased. This delayed response is known as the after effect.

SLEEP MOVEMENTS

Among the most interesting of phototropic reactions are sleep movements; though not common, they are very characteristic of certain species. Many legumes fold their leaves at night, among them being clover, peas, *Mimosa, Acacia, Oxalis* and *Cassia* (Figs. 78, 79). Numerous flowers also close at night, for example, those of *Oxalis*, *Acacia,* clover, and beans. Sleep movements in plants may be accomplished by osmotic mechanisms and not by growth reactions. This is the more plausible deduction where the movement is a daily one because it is then readily reversible.

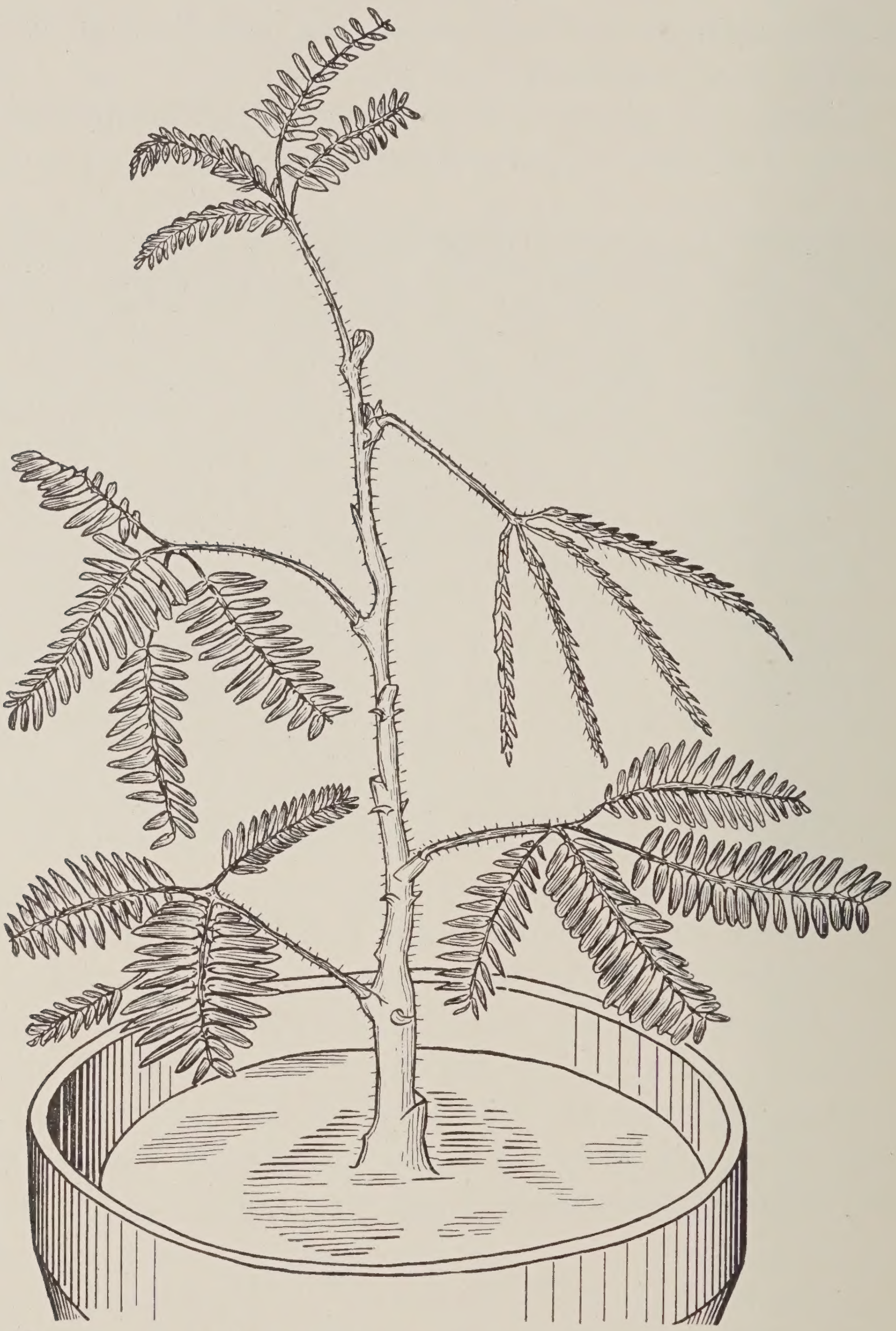

FIG. 80. Response of *Mimosa pudica* to shock; mere touching has caused the leaflets of one leaf to close and the leaf to droop. Note swelling or pulvinus at base of each petiole; closed position is also assumed at night.

Sleep movements may be of benefit, for folded leaves transpire less than open ones, and transpiration at night would be a wasteful loss of water.

IRRITABILITY

Irritability is the response of an organism to any form of external stimulus, but will be used here to imply only a reaction to mechanical shock. The classical botanical example of response to shock is furnished by the sensitive plant, *Mimosa pudica*. The leaflets fold against each other when touched (Fig. 80). The significance of this irritable response has not been discovered.

If the *Mimosa* leaves receive a strong enough shock, the stimulus travels down the petiole, closing each pair of leaflets as it goes, until it reaches the base of the petiole; there, as at the base of each leaflet, there is a swelling known as a *pulvinus*. The pulvinus is of spongy tissue and probably functions through loss of water by exosmosis, thus decreasing turgor and allowing the petiole to fall. Later on, regaining water by endosmosis and thus increasing turgor, the petiole is raised.

Burge attached weights to the leaves of *Mimosa* to see if work or exercise would make the "muscles" of the leaves less susceptible to fatigue and increase their capacity to do work, as is the case with the muscles of animals. Each day the weight lifted by the leaves as they rose from the drooping position was increased. At the end of a month, the weight an exercised leaf could lift was 40 per cent greater than that which the control leaf could lift.

Other examples of responses to mechanical shock are the closing of the leaves of the Venus flytrap and the bending of the tentacles of the sundew when stimulated by insects (see pages 280, 281). The leaves and tentacles do not move until irritated by an insect or other object. It is a curious fact that they do not respond to a single touch, but only to two successive ones.

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CHAPTER XXIV

PLANT ASSOCIATIONS

EPIPHYTES

Many plants live in permanent association with other plants. In some cases the association is a purely superficial one; in other cases it is intimate. Parasites represent the latter extreme, climbers and epiphytes the former. Plants which get no nourishment from the plants upon which they rest, provided they have no contact with the soil, are called *epiphytes*. Epiphytes are a striking feature of tropical vegetation. Certain orchids and members of the pineapple family are typical epiphytes. Usually no harm results to the tree upon which epiphytes rest. Sometimes an otherwise innocuous epiphyte may do harm only because it grows too abundantly; this is the case with the Spanish "moss," *Tillandsia usneoides,* which, when very prolific, will smother a small tree. Apparently, in a few rare instances, epiphytes make use of the decaying organic matter under the bark of the tree on which they are growing, as seems to be true of the strangling fig. The epiphyte thus becomes a *saprophyte*, a term describing plants which live upon dead organic matter, such as decayed wood. Possibly such a saprophytic stage was passed through in the evolution from epiphyte to parasite.

The relationship between an epiphyte and its support is so casual that it alone offers no physiological problem, for the same epiphyte does just as well on any other support. The Jamaican tank epiphyte, *Caraguata,* will grow upon a stone as well as upon a tree; the Spanish "moss," *Tillandsia,* and the lichen, *Usnea,* flourish as well hanging from a telegraph wire as from the limb of a tree. Epiphytes are of physiological interest not because they are party to a life-long association, but because they are adapted to an unusual environment (page 296).

PARASITES

Parasites derive part or all of their nourishment from the plant, or animal, on which they live. When but partly nourished by the host, they are said to be *semi-* or *hemiparasites*. The mistletoe is a hemiparasite, and, like most plant parasites, sends *haustoria* or special absorbing organs into the tissue of the host. These penetrate through to the xylem in the mistletoe, as it absorbs no organic food from the host, only water and salts. That this is true is obvious from the fact that the mistletoe is green and is quite capable of making its own food.

Tropical mistletoes have an interesting habit which greatly increases the spreading of the parasite. The mistletoe sends out runners along the stem of the host. From the runners at appropriate distances, shoots develop and haustoria penetrate the host, thus assuring ample nourishment and abundant propagation.

The true parasite lacks chlorophyll and must therefore obtain all its nourishment from the host. One of the best known is the dodder, *Cuscuta*. It attacks a great variety of plants and often displays luxuriant growth. It is a typical seed plant but lacks chlorophyll and roots, haustoria taking the place of the roots.

The vast majority of plant parasites are fungi, relatively few seed plants having reverted to this habit. Higher plant parasites, however, are well represented by the members of the Orobancaceae, a family including the root parasite, beechdrop, *Epiphegus*.

SYMBIOSIS

It has been stated that all degrees of relationship exist between plants. Two plants may live together with mutual benefit, without benefit or harm to each other, with harm to one and benefit to the other, or with partial harm and partial benefit. Where the benefit is in part mutual a condition of *symbiosis* exists. Where the benefit is fully mutual the relationship is known as *communal symbiosis*, and, where only one member of the partnership is benefited without harm to the other, the relationship is *commensural*.

One of the clearest cases of communal symbiosis in the plant kingdom is that existing in lichens, where a fungus is intimately and constantly associated with a one-celled alga, often the green alga *Pleurococcus*. The threads of the fungus are wrapped about the algae, affording them protection, a home as it were, in return for which the algae give food, as they possess chlorophyll and can manufacture it. The alga may live independently, but the fungus cannot live for any length of time without the alga. Furthermore, a definite species of alga is always associated with a certain fungus.

In many cases of symbiosis, it is not easy to prove benefit to both members. To know whether or not both partners benefit is of physiological interest, but that a benefit to both or either party exists cannot be assumed. In interpreting nature's ways, a reason is often taken for granted when actually none may exist. The strangling fig starts life as an epiphyte, possibly in part saprophytic upon the tree. After gaining maturity, with its roots firmly established in the soil, the fig is an independent individual. It now proceeds completely to envelop and then smother the tree which gave it a start. Reason for the strangling is evident in the benefit to the fig which now has for itself the space, the soil, and the sunlight formerly occupied by the host tree, but it is difficult to appreciate the need of sacrificing one tree for another when this could have been avoided by making the fig a terrestrial plant from the beginning. Nature often seems brutal and wasteful in her methods, but at times in her plant associations she gives superb illustrations of adjustments to needs with mutual benefit.

MYCORRHIZA

Mycorrhiza means "fungus root." It consists of a higher plant root and a soil fungus associated intimately in a symbiotic union. The term has been applied to the fungus which infects the root, to the root which is infected, to the compound organ resulting from the union of root and fungus, and to the condition or phenomenon.

There are two forms of mycorrhizae, *ectotrophic* and *endotrophic*. In the former the fungus forms a felt, mat, or mantle, outside and surrounding the root tip, and penetrates only the *intercellular* spaces within the root (Fig. 81). Endotrophic mycorrhizae are wholly within the root and enter the cells of

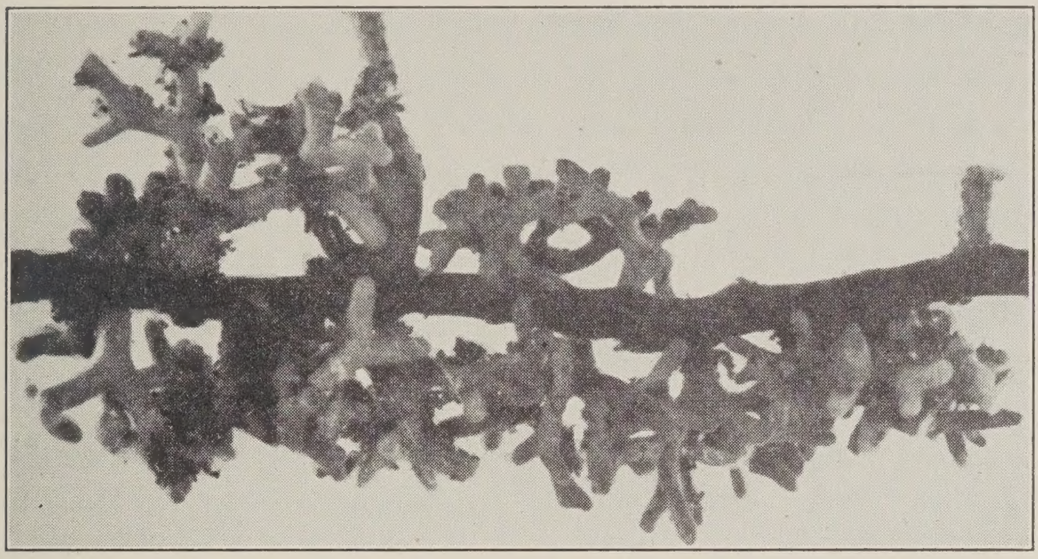

Fig. 81. Ectotropic mycorrhiza. The stubby "roots" are root within and fungal hyphae without. (Photograph, H. York.)

the tissue; they are *intracellular* (Fig. 82). There are thus two primary distinctions between the two forms, the one envelops the root and the other does not. Both penetrate the root but the

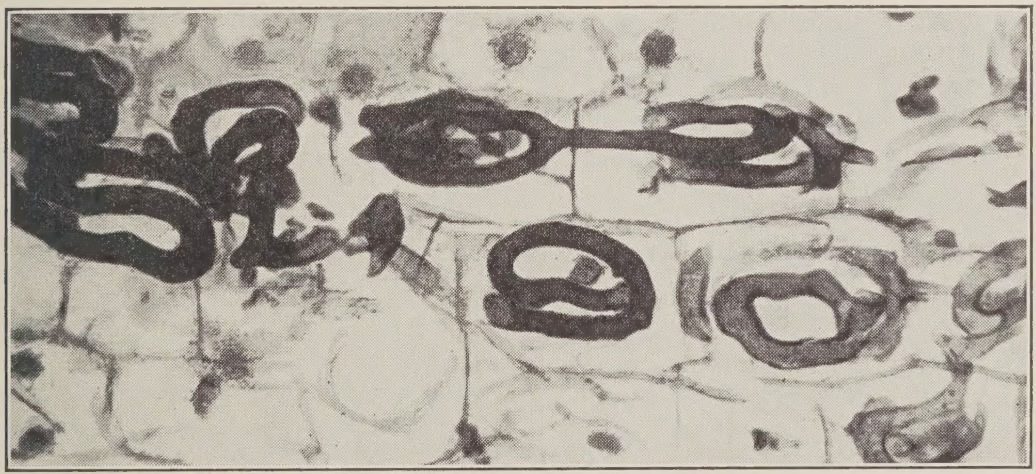

Fig. 82. Endotropic mycorrhiza in cells of root of *Liriodendron tulipifera.* (Photograph, K. D. Doak.)

one does not enter the cells, it merely ramifies between them, whereas the other lives within the cells (Fig. 82). The latter distinction is often overlooked and emphasis laid on the former;

thus one reads that the mycelia of ectotrophic mycorrhizal fungi remain on the surface of roots forming a mantle, whereas the endotrophic forms are between the root and within the cortical cells. The two errors made are the implication that the ectotrophic forms do not penetrate the root, which they must do to obtain nourishment, and that the endotrophic forms live between cells as well as in cells. The ectotrophic fungus is both within and without the root though not within the cells, whereas the endotrophic form is only within the root and within the cells. The primary cortex is the tissue involved in both forms.

Mycorrhizal roots lack the hairs that usually occur on root tips. This has led to the hypothesis that mycorrhizal fungi supplant root hairs, but the theory collapses when applied to the endotrophic forms, for they are solely within the root and send no hyphae to the exterior where they might function as absorbing structures in place of hairs. The external hyphae of the ectotrophic forms could, however, function in the place of root hairs.

Among trees, beech, oak, elm, pine, and spruce possess the ectotrophic form, whereas maple, ash, yellow poplar, and cedar possess the endotrophic form of mycorrhizae.

Most soils contain fungi capable of forming mycorrhizae on trees and shrubs.

Mycorrhizae are produced by fungi from every known order. The Basidiomycetes usually form the ectotrophic mycorrhizae, but some soil-inhabiting Ascomycetes may do so as well. These fungi can live saprophytically in the soil in the absence of plant roots. Endotrophic mycorrhizae are produced by the Phycomycetes and the Ascomycetes; sterile mycelia or non-sporulating fungi are also involved.

Up to the present time, 178 gymnosperms and 997 angiosperms are known to be mycorrhizal. In rare cases the fungus may develop both ectotrophically and endotrophically; but in most instances this is not so. When this apparently occurs, it is questionable whether the association is with two mycorrhizal fungi or with one mycorrhizal fungus and another pathogenic one.

The close association between the mycorrhizal hyphae and the root indicates that both are obligate symbionts, i.e., the parasitic condition is necessary to the full life of each. However, work by Doak indicates that mycorrhizae are not strictly beneficial nor harmful in all cases and under all conditions. For a given species of fungus and host the mycorrhizae may be beneficial to the growth of the host; in other cases there may be no benefit. No benefit does not imply an injurious effect, for the mycorrhizal fungus may be sufficiently non-pathogenic as to produce no obvious disease condition. The fungus, however, must be regarded as a weak pathogen, the activity of which is curbed by the host. Some authors are of the opinion that a mycorrhizal association is simply a controlled parasitic attack and has no mutualistic significance.

INSECTIVOROUS PLANTS

Several species of plants have devices for catching and digesting insects. The best-known examples of these insectivorous plants are the pitcher plants, *Sarracenia* and *Nepenthes*, the Venus flytrap, *Dionaea*, and the sundew, *Drosera*. In the pitcher plant, the pitcher (Fig. 83) serves both as the trap and the prison for the insect; at the bottom within the pitcher is the digestive fluid secreted by the plant. The insect enters, and once within it finds the space is too narrow for flying. On attempting to crawl up the walls, it encounters downward-pointing hairs which cover the inside of the pitcher; these prevent egress. Eventually, exhausted, the insect falls into the fluid at the bottom of the pitcher to be drowned and digested.

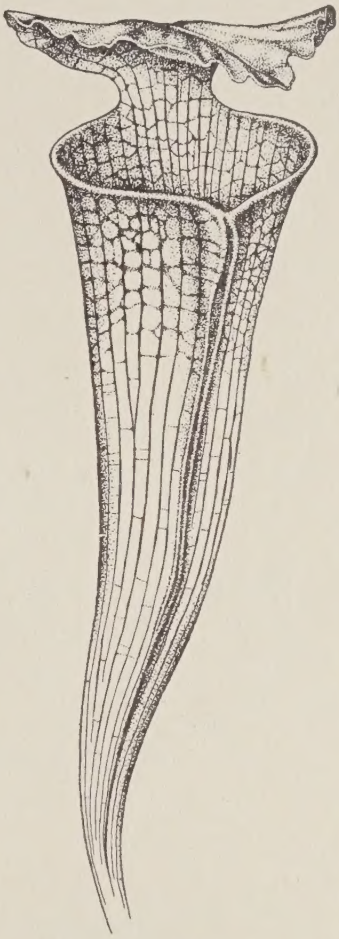

FIG. 83. Pitcher of the insectivorous plant, *Sarracenia*.

The mechanism of the Venus flytrap, *Dionaea* (Fig. 84), is more intricate. The two leaflets which form a leaf are joined by tissue which functions somewhat as a muscle. The mechanics of the motion is probably like that in the sensitive plant where reduction in pressure by the exosmosis of water closes the leaflets and increase in pressure by endosmosis opens them. A fly, alighting on the leaf surface of *Dionaea*, irritates the sensitive hairs growing thereon. The stimulus is transmitted to the "muscle" which then closes the leaflets entrapping the insect. Interlocking bristles at the edges of the leaf prevent escape. A single contact

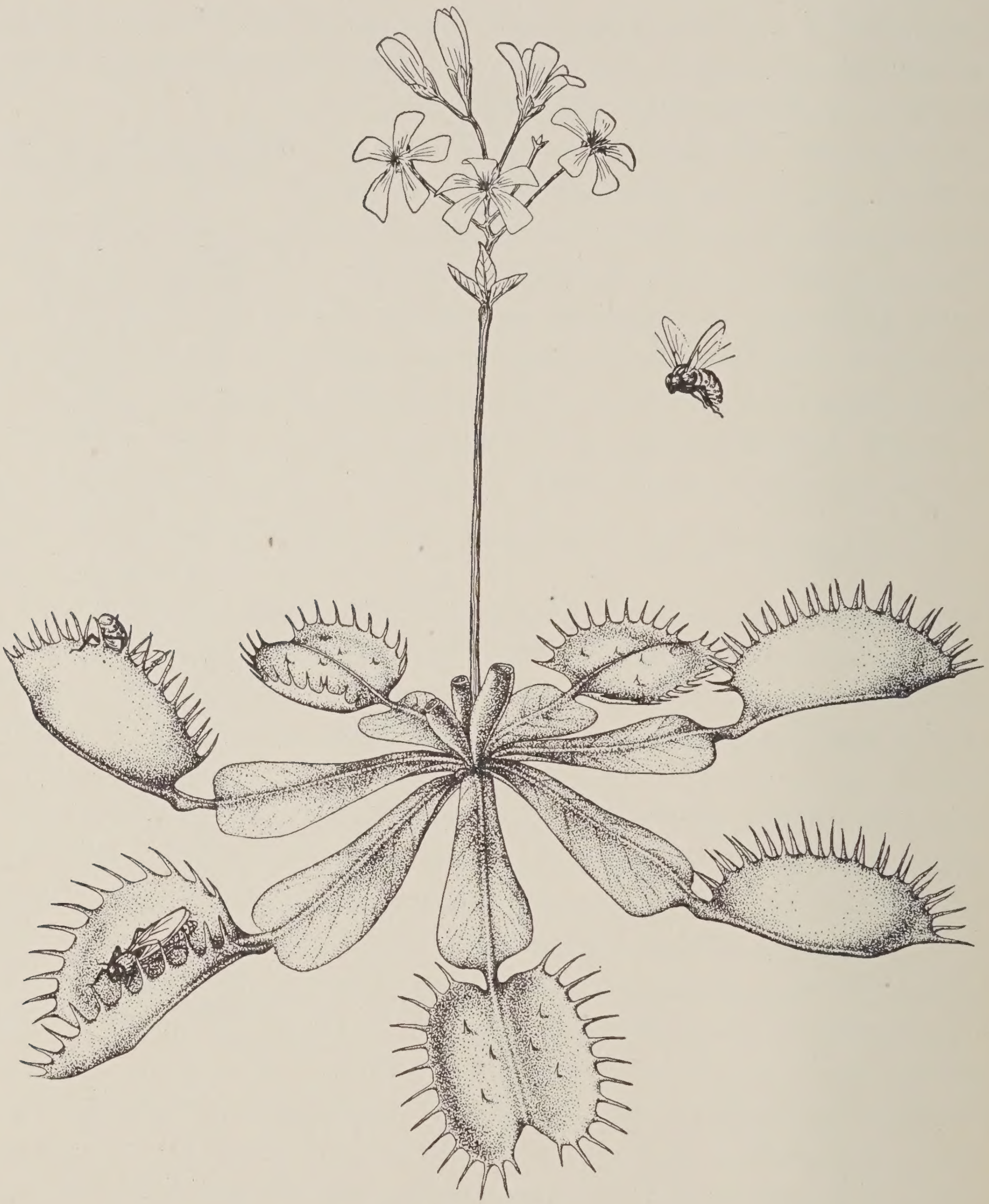

FIG. 84. The Venus flytrap, *Dionaea*.

on the sensitive hairs is not sufficient to cause closing, several being necessary. Digestion by fluids secreted by glands in the leaf follows the capture.

The sundew, *Drosera* (Fig. 85), entraps by another method. The leaf is a pad, covered with sticky tentacles. When touched several times by an insect the tentacles bend over, bringing a greater number of them into contact with the insect. Their adhesiveness prevents the victim from escaping. Digestion follows.

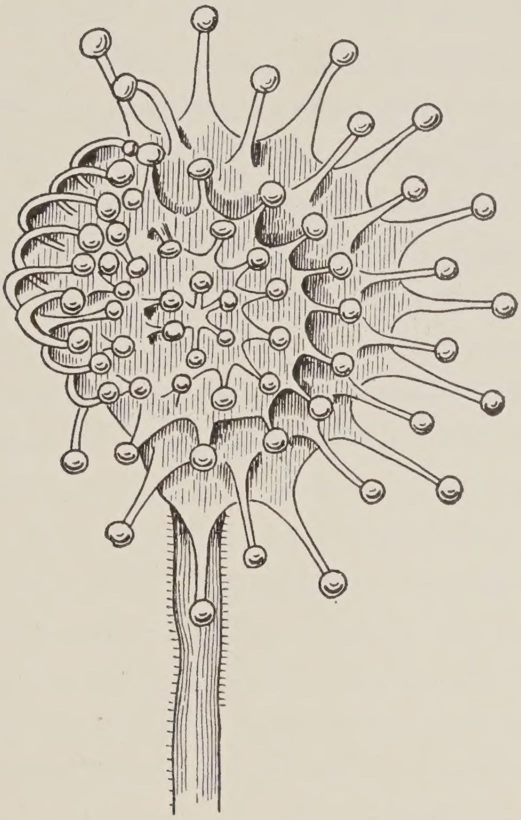

FIG. 85. The sundew, *Drosera,* with its insect-catching tenacles. (From C. Darwin.)

The digestive powers of insectivorous plants depend upon glands, the position and structure of which differ in the several types of these plants. In *Dionaea* the gland is a group of cells situated on a short stalk. In *Drosera* the tip of the tentacle is the gland; there is direct connection between the gland and the xylem and phloem. In *Sarracenia* there do not appear to be any specialized glandular cells.

Secretion of the digestive fluid apparently takes place only when the cells are stimulated by external substances of a nitrogenous nature. The ferment produced is of peptic nature associated with organic acids.

Insectivorous plants present a unique situation, that of a plant making use of organic food not synthesized by itself, in short, a carnivorous plant. That there may be a real need for organic food externally supplied is suggested in the susceptibility of the tentacles to nitrogenous matter. The tentacles respond to small pieces of meat, boiled egg, milk, and peas, as well as to insects, but, when a particle of mineral matter of the same size is placed upon them, response is very slow; thus, a piece of raw meat has been observed to cause inflection of the tentacles in five to six minutes, but response to a piece of cinder did not take place until three hours and forty minutes had passed. Sensitivity to nitrogenous food, however, is not proof of its need. Whether or not insectivorous plants need their organic diet is a question impossible to answer without extended research, which has not yet been made. The healthy condition of insectivorous plants which have had little or no organic food is evidence against the assumption that organic food is necessary. The tropical pitcher plant, *Nepenthes,* grows both at high and low altitudes in Java. Near the mountain tops, where insect life is scarce, but three or four insects will be found in a pitcher at any one time, whereas in the lowlands pitchers are often a quarter filled with dozens of insects; yet there is no observable difference in the general conditions of the plants, those at high altitudes being as healthy as the lower-growing specimens.

POLLINATION

Nature strives in every possible way to bring about cross-pollination. Plants are rarely if ever perpetually self-pollinated. Cleistogamous (closed) flowers, such as the underground fertile flowers of the violet, seem to belie this rule.

Cross-pollination is accomplished in a number of ways. Pistils in many species of plants protrude above the stamens of the flower, thus preventing the pollen from falling on the stigma. The stamens of some flowers ripen before the pistil and in this way prevent self-pollination. Wind and insects best insure cross-fertilization. Certain pollen grains possess "wings" which permit their being carried by the wind; others are coated with sticky substances which hold the pollen on visiting insects.

The most perfect adjustment between flower and insect resulting in cross-pollination is exemplified by the orchid and the bee. The mechanism was carefully studied by Darwin. The bee alights on the *labellum* or "lip" of the orchid flower and enters the nectary to suck nectar. In so doing his head comes into contact with the two pollen masses, the stalks of which have sticky pads at their bases. These adhere to the head of the bee. During the flight to the next flower, the stalks bend forward, owing to drying on the forward or windward side, so that when the bee enters the next flower the pollen sacs, which now protrude forward, strike the stigmatic surface of the new flower and thus accomplish cross-pollination. There is no way that fertilization could come about by the pollen of the flower itself; only insects seeking nectar can accomplish it.

In the *Yucca*, a liliaceous plant of the southwestern United States and Mexico, commonly known as Spanish bayonet, cross-pollination is accomplished by the *Pronuba* moth and only by it. The moth bores a hole into the ovary of the flower and there lays its eggs. It thus takes the pollen which it has acquired on visiting another flower, directly to the ovules in the ovary. When *Yucca* is grown in regions where the *Pronuba* moth does not occur, no seeds are set because there is no pollination. Here the association between plant and animal is an absolute necessity to the plant.

Although insects have first place among animals as plant pollinators, birds come in for a share, especially the humming bird. Skutch reports that flowers of *Heliconia* in Costa Rica are visited only by humming birds. Occasional cross-pollination is thus assured, but the need for it is not absolute, the flowers being capable of self-pollination. A similar example of a humming-bird pollinated plant is the tropical vine, *Marcgravia*.

CHAPTER XXV

ENVIRONMENT

The eternal question of the relative effects of environment and heredity has never been fully answered. Whereas heredity is in favor at present, and obviously will always have much indisputable evidence to support its cause as long as oaks and not roses come from acorns no matter what the environment, yet the environment often has a surprising effect on the development of an organism.

Not often can heredity and environment be sharply distinguished in their influence on growth and development, yet at times this can be done: thus, the shape of a leaf is inherited, but size, thickness, amount of chlorophyll, number of stomata, and structure of vascular bundles may vary with the amount of light and water.

LIGHT

The important role of light in photosynthesis (page 136), its effect on the development of pigments (page 141), and its full influence on growth (page 227) and plant movements (pages 268, 271) have all been considered. There are many other effects of light which, in comparison with the foregoing, appear to be minor ones, but they may often determine whether a plant is to survive or not.

Although knowledge of the cause of protoplasmic streaming is still incomplete (page 259), much work has been done on the environmental conditions affecting it. Ewart found that temperature, oxygen pressure, as well as light, influence rate of protoplasmic streaming.

Phototaxis is the adjustment in the position of microorganisms and cell inclusions relative to the direction of light rays. The zoospores of certain algae place their long axes parallel to the direction of the incident rays when exposed to oblique illumination. Desmids do likewise, and in some one-celled organisms the position assumed is always the same in reference to the polarity of the cell. Stahl was one of the first to demonstrate the phototaxis of chloroplasts. These green bodies in leaves collect along the cell walls which are perpendicular to the direction of the incident rays. Movement is regulated not only by the direction of light but also by its intensity. Chloroplasts, in intense light from above, line the vertical walls of the cells, and in weak light take up their positions on the horizontal walls (Fig. 72).

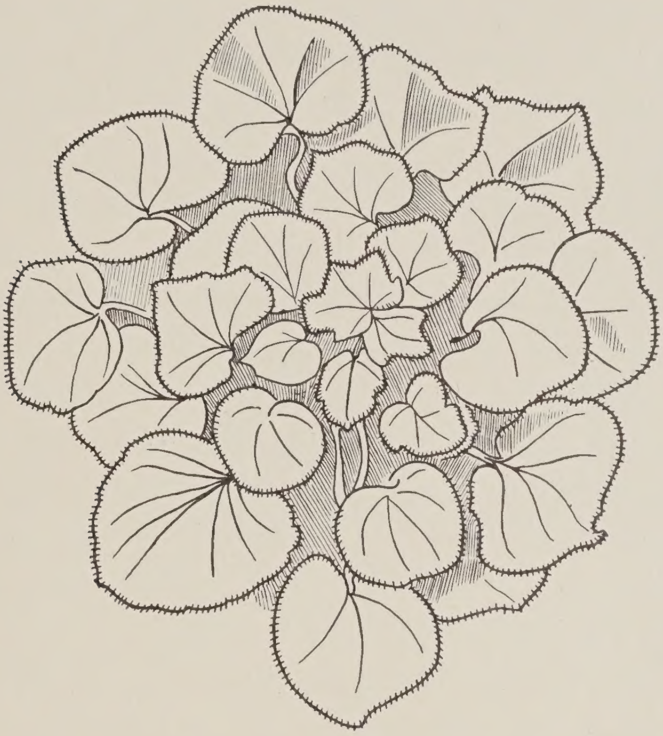

FIG. 86. Leaf mosaic of *Saxifrage*, showing perfect leaf distribution for maximum illumination.

Leaf arrangement is little affected by light because the plan is already established in the bud before light can exercise an influence; but, though predetermined, it has probably become established through the ages because of the reaction of leaves to illumination.

Leaf mosaics are an expression of the "attempt" of plants to make the most of available light. From the point of view of illumination efficiency, the leaf arrangement is often so perfect that if evolution has been by the trial and error method with the elimination of the unfit, the result is as good as it could have been through purposeful and directive development. Types of leaf arrangement with maximum exposure to light are numerous, among them being leaf mosaics such as are found in rosettes (Fig. 86), helicoid arrangements, and the leaf distribution of the fir (Fig. 60).

Some species of plants change the position of their leaves from the horizontal to the vertical in protection against excessive sunlight; thus, leaves of the dogbane, *Apocynum androsaemifolium,* are horizontal at night and early morning, and vertical at midday.

Although the foregoing leaf arrangements are typical of the plants having them, and once established are not easily altered, yet certain of them may, under extreme conditions, become so badly disarranged as to give the appearance of a different kind of plant. Balsam fir in windy places may resemble a spruce, in that its leaves, normally coplanar, come to lie all around the twig.

In addition to phototropic leaf movements (page 269) there are leaf responses to different light intensities. The leaflets of *Robinia* are horizontal in weak light but present their margins to the incident rays of intense light.

Sun and shade leaves illustrate the effect of light on leaf development. Photosynthesis is greatest in the sun, and photosynthetic tissue is more highly developed in leaves exposed to the sun than in leaves in the shade. The palisade tissue of shade leaves is of a single layer in contrast to the several layers in leaves exposed to the sun. The direct effect of sunlight is probably not the cause of the greater development of the palisade tissue of sun leaves, nor is the rate of photosynthetic activity the likely determining factor. Evaporation rate is probably of greater influence.

As strange as it may seem in view of the tremendous importance of light to plant growth, it is yet true that light may have a depressing effect on growth. There are records of more rapid growth at night than in the day. Numerous attempts have been made to explain this, such as, light hastens senescence, stimulates the production of a growth inhibitor, or retards a cell's response to a growth substance. E. S. Johnston concludes that light of high intensity has a destructive or inactivating action on plant-growth substances.

The rate of plant growth is influenced by light in another way; thus, the height of a dandelion is determined by the height of the surrounding grass because the grass determines the height to which the dandelion must grow in order that its flower will be fully exposed to light. If the grass is cut, the flowers are formed on shorter and shorter stems.

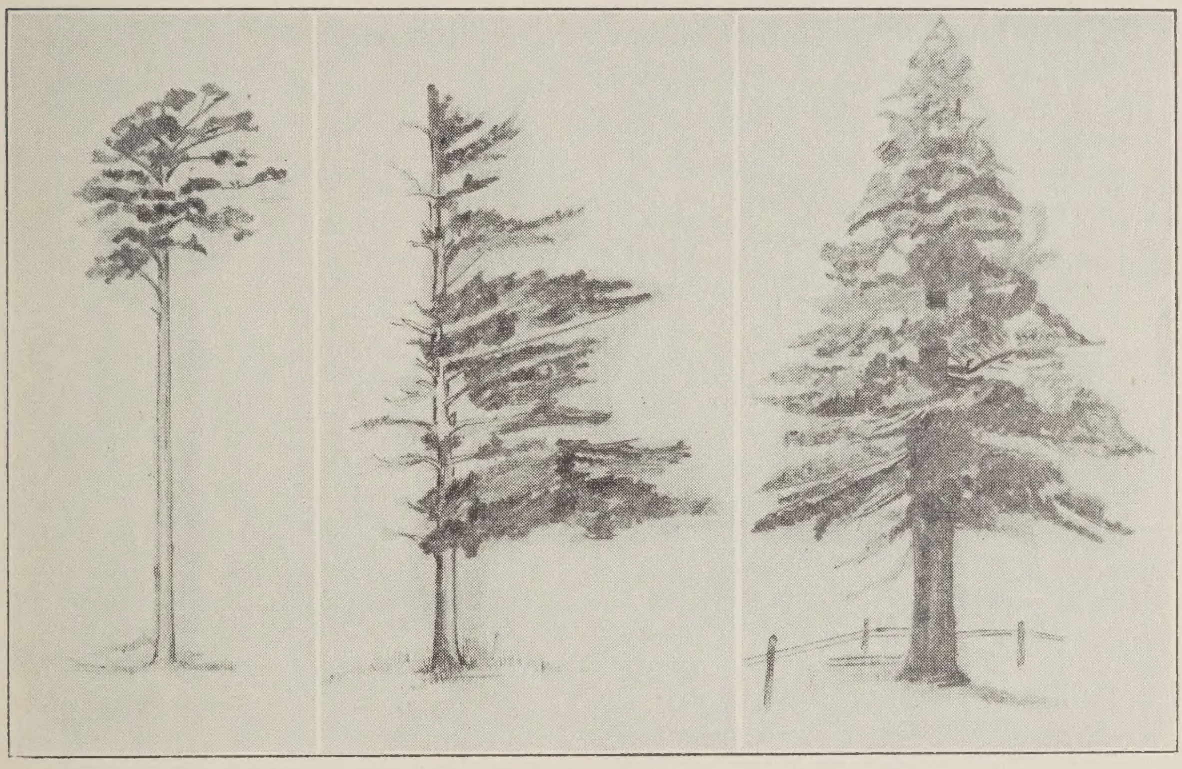

FIG. 87. Effect of light on tree form: left, within the forest; center, at edge of forest; right, alone.

Light influences the shape of trees by stopping the growth of limbs, often producing very symmetrical shapes. The perfect cone of the blue spruce is the form best suited to bring as many of the leaves as possible into the light. Trees growing within a dense forest have a long branchless trunk. The same tree on the edge of the forest is asymmetrical, and, when standing alone, fully exposed to light on all sides, it would hardly be recognized as the same species as its brother in the forest (Fig. 87).

The optical design of cells represents one of the neatest adaptations of a plant to its light environment. The classical case of this is the moss, *Schistostega osmundacea* (Fig. 88). It is usually found in shallow caves where the light is poor. Its cells are excellent optical systems. Each cell of the protonema is a lens, at the base of which the chloroplasts lie (Fig. 88B). Light enters at the top and is focussed on the chloroplasts. Thus is the little light available concentrated for photosynthesis.

One of the most startling effects of light on plants has been revealed by the work of Garner and Allard in a field of investigation now known as *photoperiodism*. By controlling the time of exposure of a plant to light, Garner and Allard were able to decrease or increase the age at which a plant reaches sexual maturity. The field aster, which commonly requires four months (May to September) to reach maturity, was made to bear flowers within a month after germination by decreasing the time of exposure to daylight (Fig. 89). Still more remarkable was it that these same plants, on being restored to normal light exposure, did not complete their life cycle by dying after flowering, as they would have done in the field, but developed new axillary branches and flowered a second time in September. Increasing the length of exposure to light has the opposite effect; flowering may be postponed until the following year.

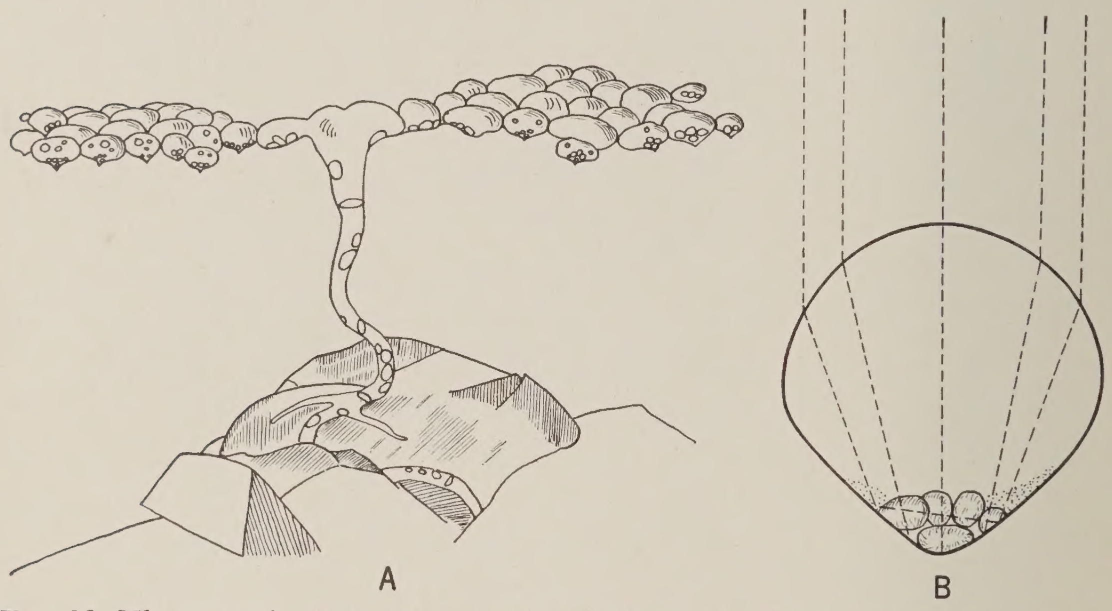

Fig. 88. The optical cells of the cave-inhabiting moss, *Schistostega osmundacea;* the cells of the protonema are lens-shaped.

The simple rule that favorable conditions tend to prolong vegetative growth whereas unfavorable conditions tend to bring on reproduction might be applied here to explain the hastening of flowering by shortened exposure to light and the delay in flowering because of increased exposure to light. The rule would work very nicely were it not for an additional fact discovered by Garner and Allard, namely, that there are both short-day plants and long-day plants, and what a given time of exposure to light will do for the one is not at all the same as it will do for the other. The photographs in Fig. 90 show how true this is.

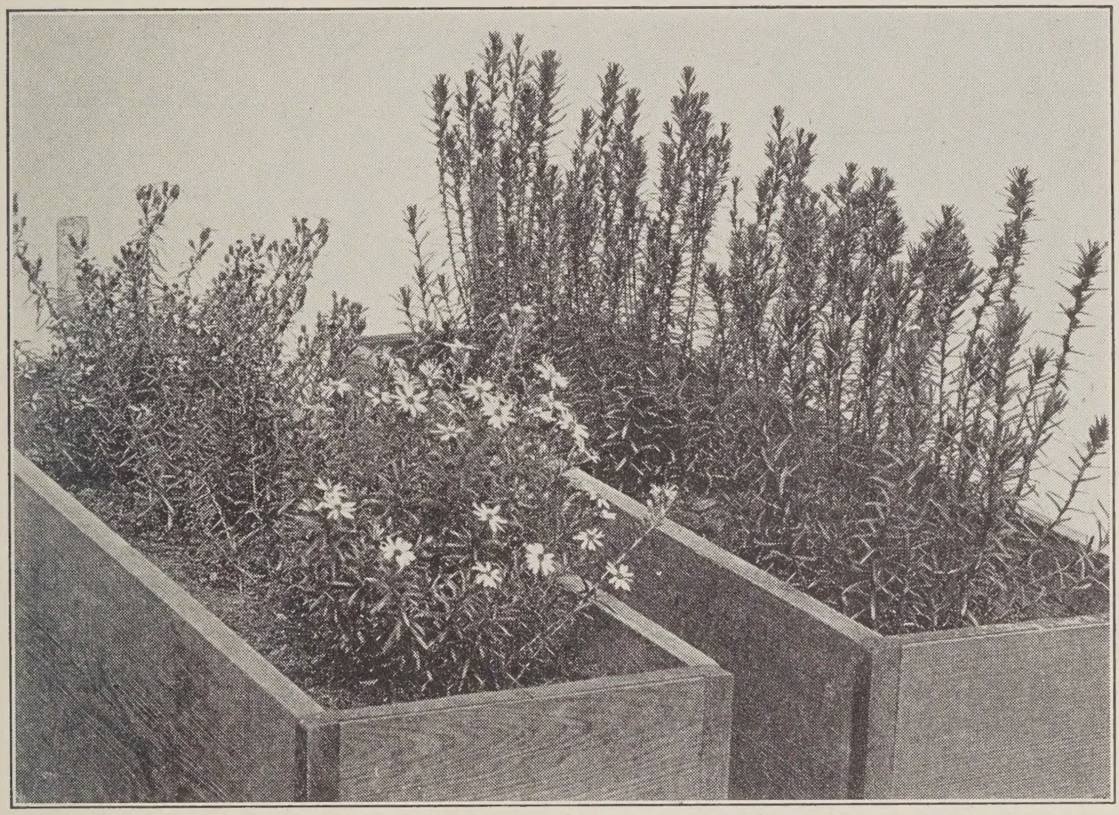

Fig. 89. *Aster linariifolius:* plants in box at left were exposed to light from 9 A. M. to 4 P. M. daily; they were in full bloom when photographed June 24. Plants in box at right were left out of doors during the test; they showed no indication of flower heads when photographed June 24. (Photograph, W. W. Garner; Jour. Agr. Res., 18.)

Intermittent illumination gives interesting results showing that the shorter the interval the closer the approach to normal growth (Fig. 91).

Again and again evidence proves that plants are not alike in their needs and their behavior: *Valonia* stores potassium; its cousin, *Halicystis,* stores sodium; some species of plants, e.g.,

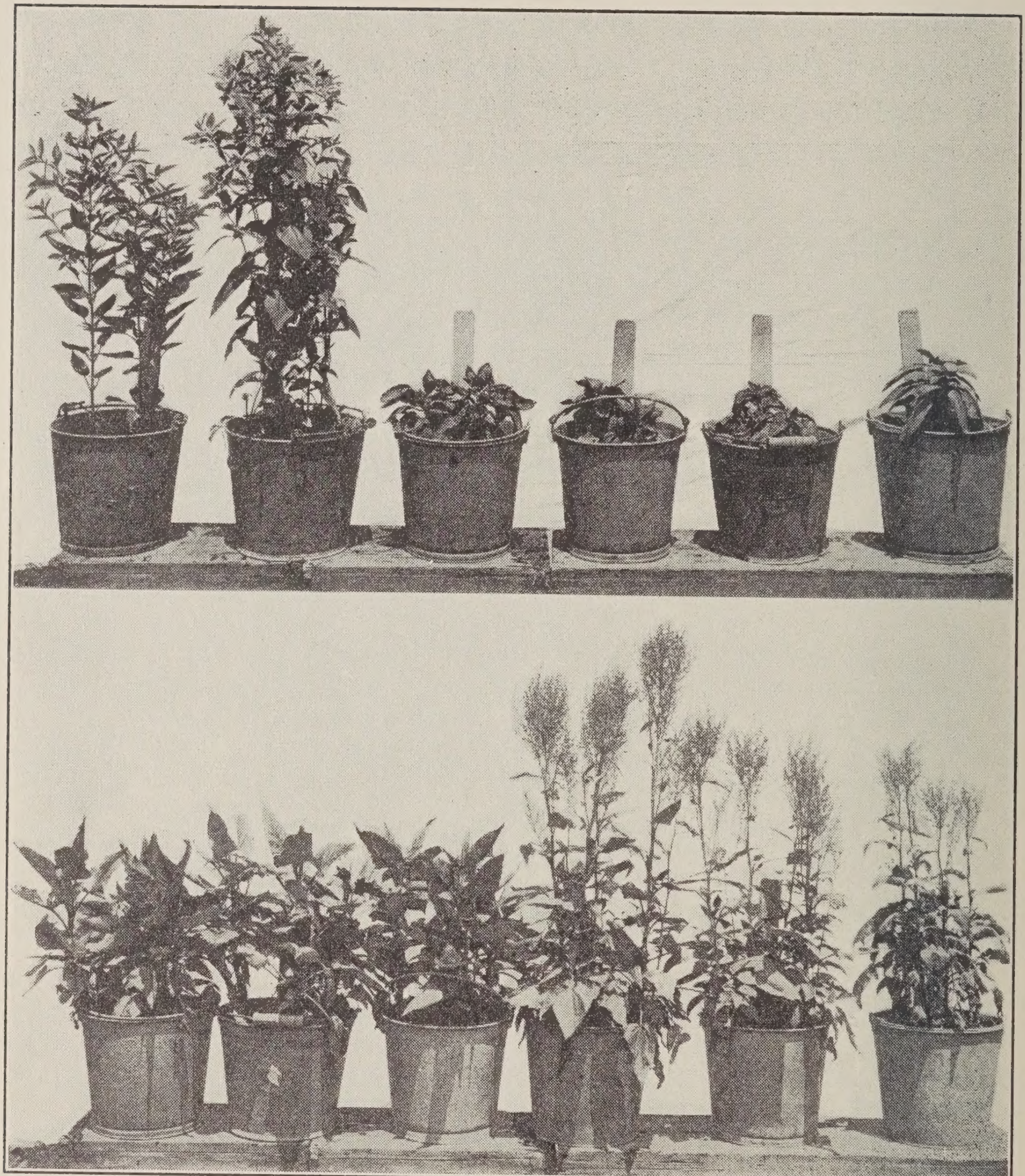

FIG. 90. *Steironema,* a long-day plant; below, *Rumex,* a short-day plant. Left to right, the light exposures were: full summer day of 14 to 15 hours; darkened 10 A. M. to 2 P. M.; 12-hour day; 10-hour day; 8-hour day; 5-hour day. For the long-day type, *Steironema,* the critical light period for flowering lies between 12 and 14 hours; with periods below the critical the plant tends to remain in the leaf rosette stage. For the short-day type, *Rumex,* the critical period is about 11 hours; with longer day lengths the plant develops indeterminate vegetative stems. Midday darkening produces no formative effect in either type of plant. (Photograph, W. W. Garner; Biol. Effects of Radiation, Vol. 2.)

Rhododendron, do best in acid soil; many of the legumes do best in alkaline soil; the field aster is forced early into flower by a short day whereas *Steironema* is forced into flower by a long day. Much futile argument would be avoided if the individuality of plants received half the recognition given the individuality of people.

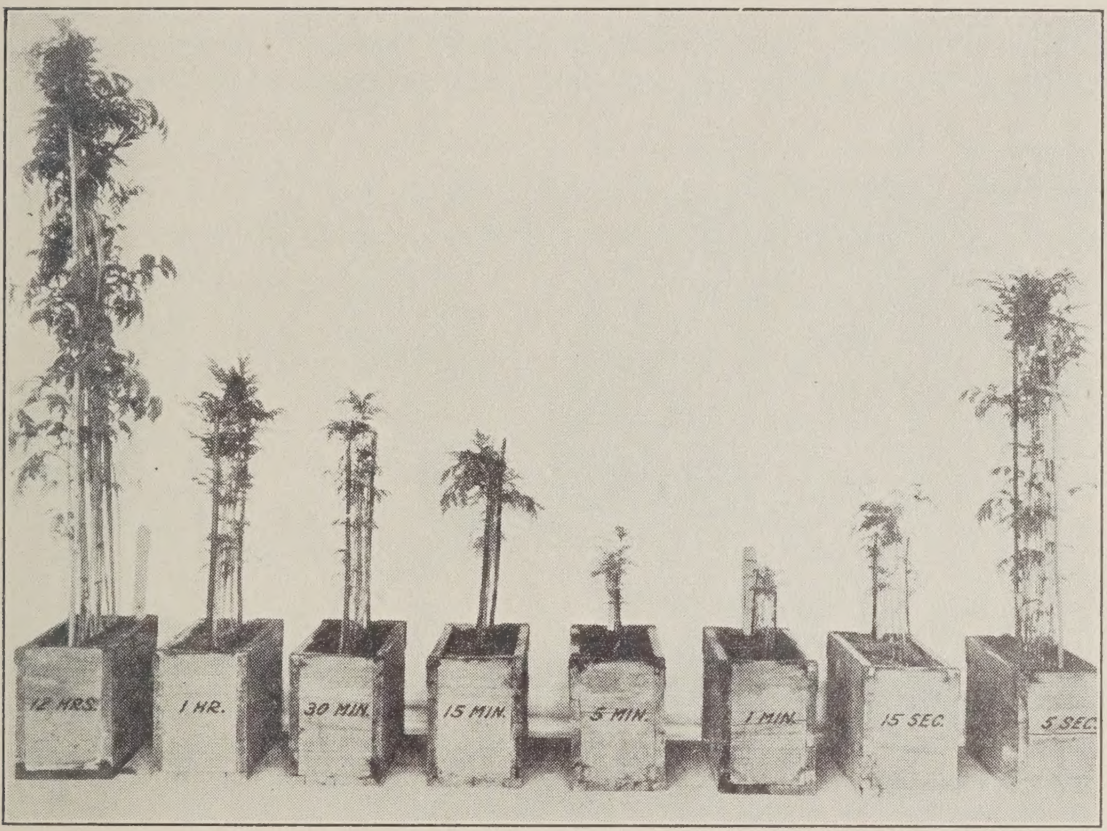

Fig. 91. *Cosmos sulphureus* grown with equal alternations of light and darkness, ranging from twelve hours to five seconds. With decrease in the intervals of light and darkness there is progressive decrease in height, size, and weight of the plants, and increase in etiolation and attenuation till the one-minute intervals are reached. Further shortening of alternations causes improvement in growth and appearance of the plants. All intervals from one hour downward are unfavorable for flowering. (Photograph, W. W. Garner; Jour. Agr. Res., 42.)

Experiments in photoperiodism are likely to find a practical application in agriculture through advancing the time of fruiting of grains and other horticultural plants, and in determining what crop plants are suitable for growth in the tropics where the days and nights do not differ by more than an hour, and in the arctics where the summer days may be some twenty hours in length with twilight persisting through the remaining hours.

Anderson has observed the effect of light on the layering of cellulose in the cotton fiber (Figs. 47, 48, 49). He finds that the normal layering in cotton fibers (Fig. 92, left), due apparently to a day and night rhythm, is eliminated by continuous exposure of the cotton plant to light (Fig. 92, right). Thus is the internal as well as the external anatomy of a plant determined in part by light.

In all studies on the influence of light, it is necessary to remember that white light is not homogeneous but consists of many colors, each of which may have its individual effect. This is true in the case of photosynthesis, where green light has little or no effect, whereas red and blue are most effective, though not equally so (page 137).

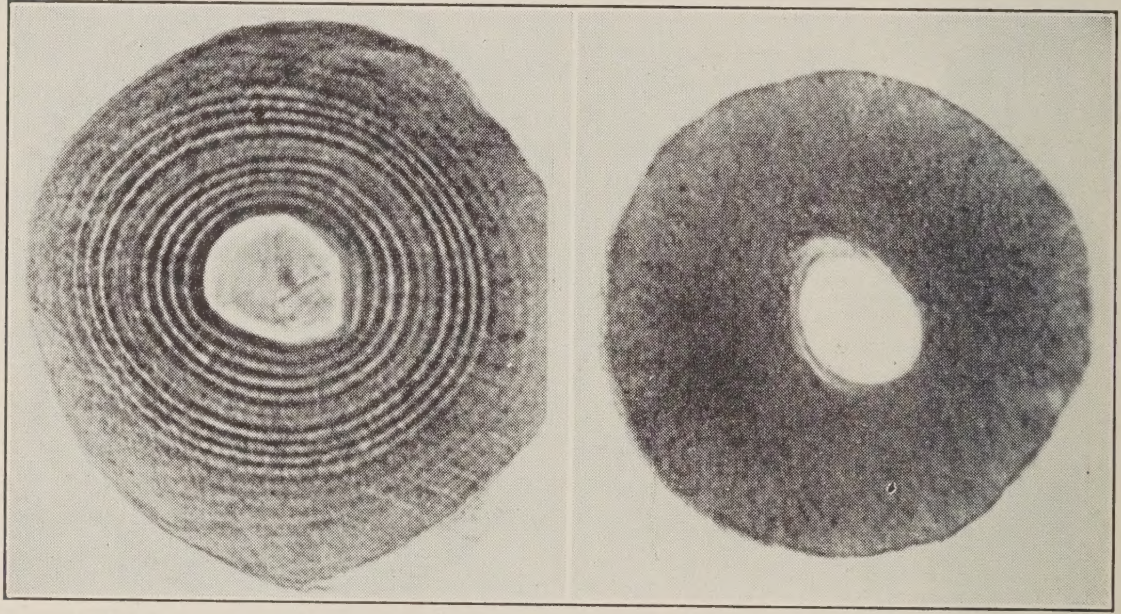

Fig. 92. Effect of continuous illumination: (right) on the layering, (left) in the cellulose wall of cotton fibers. (Photograph, D. B. Anderson.)

The curve in Fig. 41 and the absorption spectrum in Fig. 39 make this clear. The regions which show optimum growth are red at 6500 A. U. and blue at 4400 A. U.

Light beyond the visible spectrum does not appear to play much part in photosynthesis, but it has its influence on other plant activities. E. S. Johnston has shown that infrared added to visible light causes plants to increase in size and weight, but to lose in health—yellowing and occasionally death occur—and J. M. Arthur has demonstrated that pigmentation in apples is increased by ultraviolet whereas infrared has a detrimental effect.

The influence of ultraviolet light is of particular interest in view of its harmful as well as beneficial influences on man. The damaging effect of ultraviolet light is familiar in sunburn; its curative powers are greatly valued in the treatment of a number of diseases, particularly rickets. Why these apparently conflicting statements are both true is shown by the results of careful research which permits screening out certain rays and exposing the plant or animal to only a narrow band in the ultraviolet. This was done by F. E. Meier who placed cultures of the alga *Chlorella* in the ultraviolet spectral lines of a quartz mercury-vapor lamp. The points of greatest toxicity were selected and characterized on the basis of maximum lethal sensitivity and maximum virulence. The former describes the certainty of the lethal action; the latter the quickness of it. An analogous case would be the effects of cyanide and radium on man. On the basis of minimum lethal dose or sensitivity, radium is more toxic than cyanide; but, on the basis of virulence or time required for lethal effect, cyanide is more toxic than radium. In the case of the effect of ultraviolet on the alga, *Chlorella,* maximum lethal sensitivity, determined by dosage, is at 2600 A. U., whereas virulence, determined by time, has a maximum at 2323 A. U.

MOISTURE

Reference has already been made to the numerous ways in which plants maintain an adequate water supply and protect themselves against excessive loss of water (pages 90, 91). These are all adaptations to environment based on moisture conditions. The desert plant must conserve water, and it does so in special water-storage tissues, whereas the rain-forest plant has no such tissue, drought being unknown to it.

Adaptations to moisture conditions are very efficient in those bromeliads which form "tank" epiphytes. Epiphytes have only rain and atmospheric moisture as a source of water. Many bromeliads catch and retain this water in the "tanks" formed by the leaves.

A striking response of a plant to its moisture environment is that of the tropical pitcher plant, *Nepenthes,* which grows as a rosette and develops pitchers when on wet, marshy ground, but grows as a vine 20 to 30 feet long and forms no pitchers when on dry ground.

Roots are especially responsive to their moisture environment. In addition to their hygrotropic responses (page 265) they show morphological differences with change in moisture environment. The roots of certain land plants are replaced by other roots of different structure when the plant is taken from the soil and placed in water. Conversely, if certain land plants are grown from the beginning in water and then transplanted to the soil, they die. Apparently, water roots are not adapted to the absorption of the hygroscopic (colloidal) water of the soil.

Aquatic plants possess some very striking devices which adapt them to their environment. Coiled leaf stalks stretch without breaking and thus permit floating leaves to rise and fall with the waves without submerging the leaves. Particularly interesting are the mechanisms for pollination in aquatic plants.

Vallisneria is an aquatic plant which lives wholly submerged except at the time of pollination, when the female flower bud is raised to the water surface on a spirally wound peduncle; there it floats and blossoms. At about the same time the male flower bud is raised on a separate stem but not to the water surface; midway, growth stops, and then the bud separates from its stalk and, buoyed up by a pocket of air, rises to the surface, where with stamens spread apart, and wholly independent, floating about like a little boat, it bumps against the female flower, pollinating the stigma.

The flora of an entire region is often determined by prevailing moisture conditions. Deserts harbor cacti which are well suited for a xerophytic existence because of their water-storage tissue. Wet ravines are often characterized by a prolific growth of ferns —temperature is also a factor here. Tropical mountains are wettest at about two-thirds of their height where clouds cling. The moss flora on such a mountain slope is sharply delimited into zones determined by moisture. Near the mountain base few mosses exist. Two-thirds of the way up, say at 6000 feet, where the clouds hang close, mosses are abundant and the prevailing type of moss is a pendant one; long streamers swing from limb to limb or hang free in the air. At 8000 feet, where the slope is exposed to desiccating winds, festooning mosses are replaced by tight and compact moss cushions, veritable sponges. At 9000 feet, on the wind-swept summit, not even a moss sponge can hold water long enough to survive, with the result that no mosses grow, their places being taken entirely by lichens.

WIND

Wind has a pronounced influence on plant form. At high altitudes trees assume a twisted and gnarled shape due in part to the mechanical effects of wind. Such trees are known as *Krumm-* or *Kneeholtz* (crooked or kneewood), and are common at the tree line on mountains the world over. Grotesque forms of trees are produced by wind along seacoasts and on high plateaus where the trade winds are in one direction. The tree may lie parallel to the ground with a full right-angle bend

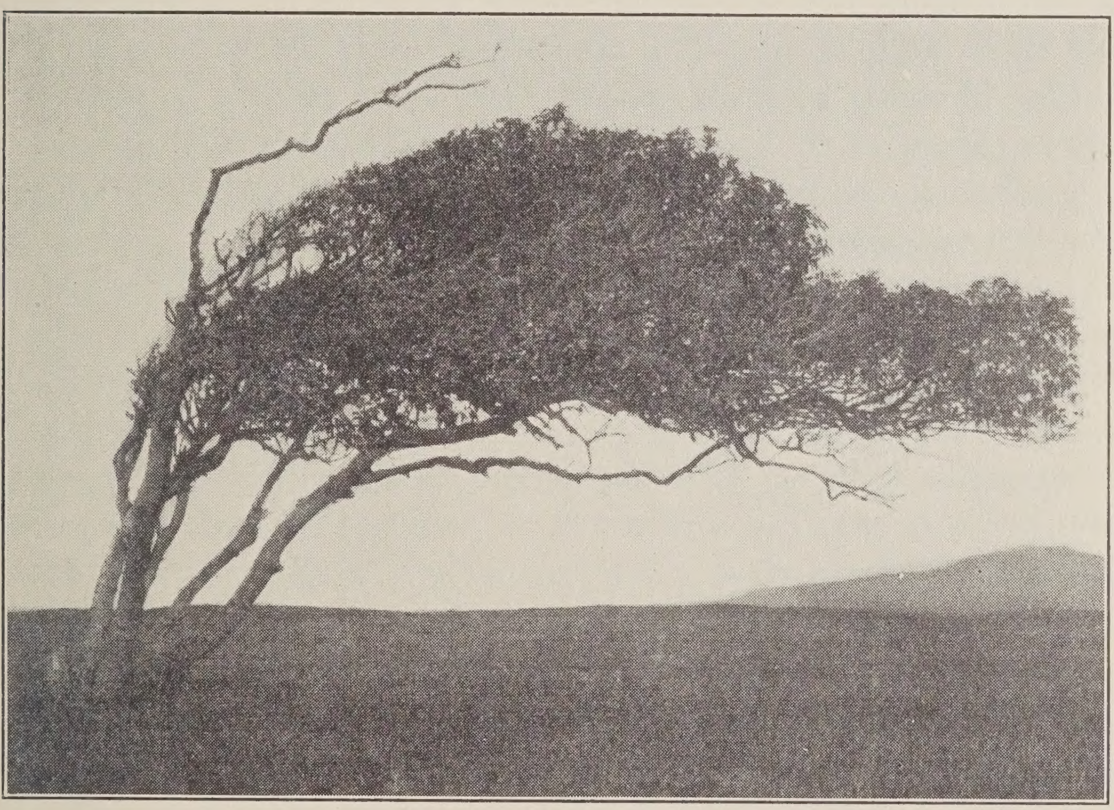

FIG. 93. Effect of the trade wind on a lone pua tree (*Osmanthus*). This valiant sentinel stands on the north plateau of Lanai, Hawaiian Islands. (Photograph, Bishop Museum, from R. Fosberg.)

in its trunk (Fig. 93). The weird shapes of the Monterey (California) cypresses are due to the wind. The controversial question arises whether the plant "meets" the situation caused by the wind through a modification in structure, or whether the change is a purely passive one.

EPIPHYTES

Epiphytes, or plants growing on other plants without getting any nourishment from them, are specially adapted to their form of life. Tank epiphytes, already referred to, become, through the arrangement of their leaves, water-holding receptacles. Epiphytic orchids have developed other methods for making possible their mode of life. The air roots of orchids are adapted for taking in water by means of an outer layer of cells or *velamen*, so modified as to become an efficient absorbent. In taking in atmospheric water, the air roots of orchids absorb, in solution, the mineral substances which have collected on the surface of the roots from the dust of the air. Thus they are able to live without contact with the soil.

Extraordinary modifications in structure, with corresponding adaptations to meet an extreme type of epiphytic life, occur in the Spanish "moss," *Tillandsia usneoides*. This plant has become so greatly changed as a result of its habitat that little more than the stem remains. Roots have entirely disappeared, leaves are reduced to tiny scales, and flowers are both inconspicuous and unnecessary; propagation can be abundantly accomplished by the wind and by birds carrying small pieces of the plant from one support to another. Seeds are produced, but few of them fall into places favorable for growth.

THE RADIATION ENVIRONMENT

Plants and animals are subjected to numerous types of radiation, to heat, light, electricity, radium emanations, and cosmic rays. All probably influence development, but the extent of their influence is known only in a few instances. The effects of heat and visible light have been discussed several times (page 285).

Beyond the violet of the sun's visible spectrum lies the ultraviolet, and beyond the red or long-wave end of the spectrum lies the infrared. The extent of the effect of these invisible forms of radiant energy on plants is not fully known. Organisms in general are benefited by ultraviolet light of certain wave lengths, as in the case of rickets in children, but harmed by ultraviolet light of certain other wave lengths. Lower forms of plant life, such as bacteria, may be killed by ultraviolet light.

Ultraviolet is generally thought not to be consequential in activating photosynthesis (page 137), but its deadly effect on bacteria and protozoa, and its beneficial effect on higher animals, force one to conclude that it is by no means without influence on higher plants (page 292). The same may be said of the infrared.

The effect of electricity and allied forces on plant life has been investigated from various points of view. It has been suggested that mitosis might be due to magnetic forces, for the mitotic figure appears to be the result of forces emanating from two poles. This hypothesis led to an attempt to disturb the normal process of cell division by placing a dividing animal egg in the field of a powerful electromagnet; but nothing happened —the egg continued to divide in the normal way. However, hypotheses of the movement and orientation of chromosomes based on magnetic forces continue to be advanced. To be sure, magnets, when floating on corks on water, have been found to assume the same relative positions as do chromosomes; but magnetic theories of mitosis and other vital processes have little experimental or theoretical support.

Electroculture is the growing of plants under the influence of static electricity. Plants out of doors with their roots in the earth and their tops in the air are continually being traversed by minute currents of electricity, because the air is electrically charged with respect to the earth. It is therefore reasonable to suppose that an increase or decrease in potential between earth and atmosphere would affect the growth of plants. English investigators have obtained evidence which they interpret as demonstrating that increased plant growth follows electric excitation of a certain intensity and duration when supplied from a charged network suspended above the plants. However, L. J. Briggs and others, working over a period of ten years, treated plants in the field and greenhouse under very carefully controlled conditions, with only negative results throughout. It is possible that the differences in results between American and English workers are due to the fact that, if electricity is a substitute for light, an additional supply of it in England would be of advantage to the plants; but in the United States, where the average daily sunlight is in excess of the plants's needs, more energy in the form of a substitute would be of no benefit. The damp atmosphere of the British Isles may also be a factor.

The possible effect of electricity on plants brings up the allied question of the likely presence of electrical forces in plants. There can be no doubt that there are differences in levels of free energy between tissues. The fact that tissues differ in salt concentration, in acidity, and in intensity of chemical reactions is proof of differences in levels of free energy, but that this energy is necessarily electrical cannot be assumed.

If a difference in potential between two points is measured, all that can be maintained is that there exists a difference in level of free energy; but the energy thus liberated may be electrical, mechanical, chemical, or thermal. The difference in energy level is expressed in terms of potential because the potentiometric method was used for measuring it. It is not proved that an actual flow of electric current takes place in tissues. On the other hand, as the two regions of different potentials are connected within the plant body by electrolytic fluids, the possibility of a transfer of ions, such as takes place within a galvanic cell, must be admitted. Electrolytic conduction is ionic, in distinction from metallic conduction, in a wire, which is electronic, and as ions are present in the body solutions of plants, electrolytic conduction probably takes place. A study of the effect on plants and animals of the radiant energy given off by heavy metals has yielded unexpected results. Mutations have been produced in tobacco and in fruit flies subjected to radium emanations, both in the laboratory and from natural deposits. It has been suggested that the course of evolution has been in part determined by radiation from the earth. However, this entire question may be viewed in a different light. In the first place, the reaction of protoplasm to a damaging effect is hardly a mutation. The cell is alive and reacts to radium emanation. Abnormalities so produced may be, but are not necessarily, mutations. Furthermore, as the organisms produced by radium treatment have in no instance been better than the parent, this is evolution only in the sense of change and not improvement.

While the protoplasm of some organisms appears to be very sensitive to radium emanations, other forms show extraordinary resistance. The plasmodia of slime molds will approach a radium needle and stop within a centimeter of it (Fig. 94, left); then, after a pause, proceed and pass over the needle, entwining it for a time (Fig. 94, right). They cannot, however, endure the radium indefinitely and soon retreat, only to recover and advance again.

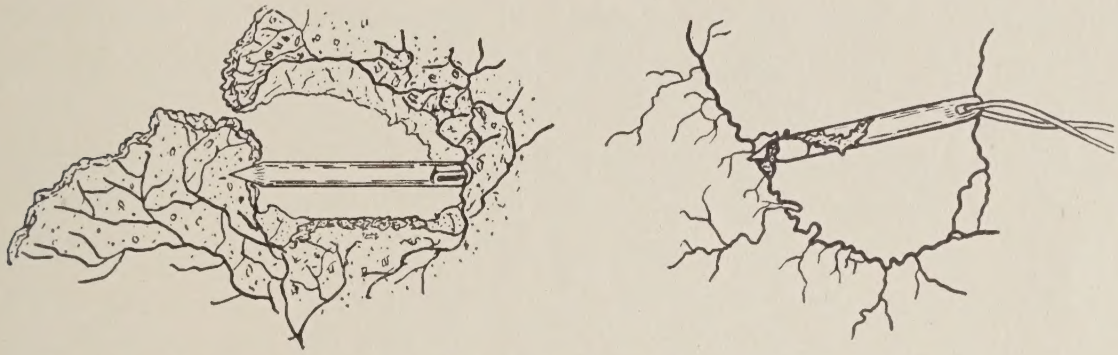

FIG. 94. Protoplasm approaching (left) and entwining (right) a radium needle. Note that the protoplasm stops for a time at a uniform distance from the radium needle (left).

Plants respond readily to X-rays, very high dosages killing them. M. Russell found that roots of *Zea mays* treated with X-rays (1500 r), applied when the primary root is one centimeter long, cause the secondary roots to appear later than they normally would. As larger doses are used, the first secondary roots appear farther and farther from the seed. More extraordinary than the retardation in appearance of the secondary roots is their continued growth *within* the primary root, both upward and downward, parallel to the length of the root (Fig. 95).

Disturbing the winter's rest of plants has been attempted by various means. F. Weber used X-rays. His work had its origin in some experiments by Hans Molisch, which showed that radium emanations would end the resting period of winter buds and bring them into leaf. As the alpha rays were screened out by glass, the beta or gamma rays were probably responsible. Weber continued the work, using X-rays, and found that they caused resting buds of *Syringa* to open prematurely. A too heavy dose caused the growing buds later to fall.

As for cosmic rays, nothing definite can be said, but they may well have an effect on living matter.

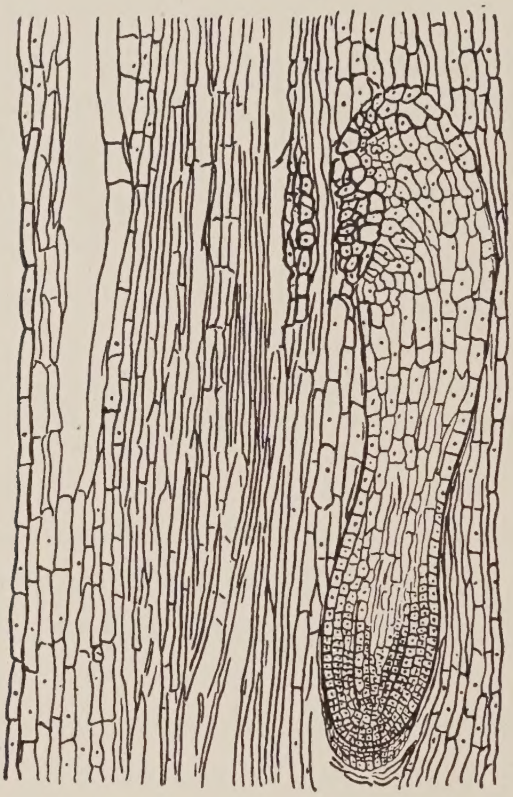

FIG. 95. Direction of growth of a lateral root *within* the parent root as a result of X-ray treatment. The small root does not emerge as it normally would. (From M. Russell.)

Protoplasm is sensitive to its radiation environment; it may also be a source of radiation. Many animals give off heat, and some few radiate light. The ray fish and the electric eel give electric shocks of sufficient strength to be felt simultaneously by a number of human beings, or to blow out a six-volt bulb. It has been suggested that living matter radiates ultraviolet light in sufficient quantity to affect the rate of growth of certain tissues and organisms nearby. The experimental results supporting this suggestion have been severely questioned, but, although the work may be at fault, the hypothesis that protoplasm radiates energy of a kind cannot be easily refuted. Every chemical reaction involves a transfer of energy which may manifest itself in the form of heat, light, or an electric potential. It is probable that protoplasm which contains the radioactive elements, potassium and rubidium, and is the seat of an almost infinite variety of chemical reactions, should radiate energy of more than one form.

Man is often ignorant of phenomena of common occurrence, sometimes owing to failure to observe and sometimes to inability to detect. Thus, the radiation of heat by plants is little known, for it is generally assumed that plants follow the temperature of their surroundings; but plants radiate sufficient heat to melt the snow about them. This is illustrated in some alpine plants, e.g., *Erythronium parviflorum* and *Caltha leptosepala*, in America, and *Soldanella alpina* and *Helleborus viridis* in Switzerland, which melt their way through snow, forming a well of water about themselves.

Growing points usually produce more heat than mature parts. Inflorescences in particular generate heat, which may, in the case of palms, cycads, and aroids, result in their temperature exceeding that of the air by 10°C.

Thus plants actually radiate a form of energy not ordinarily attributed to them, and may give rise to other forms of radiation of which man is as yet ignorant.

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CHAPTER XXVI

THE LIVING AND THE NON-LIVING

There arises in the mind of every thinking person the question, what distinguishes the living from the non-living? Any attempt to make such a distinction must, in the present state of knowledge, be in the nature of a philosophical argument, granted that an argument exists. The man of science who views physiology in a purely mechanistic or materialistic way holds that philosophy has no right whatsoever to enter into the discussion, for to him the distinction between the living and the non-living is a matter of complexity in chemical constitution and physical organization. This assumption is in itself a philosophical concept, for it cannot be proved experimentally. Those who hold this view are known as *mechanists* or *materialists*, and those of the opposing school of thought are *vitalists*. The vitalist regards life as the expression of a force that lies beyond the analytical powers of the intellect.

The discussion could not continue were the vitalist right, for a *vital force* is outside the world of physics and chemistry and therefore beyond experimental investigation, beyond analysis, and beyond measurement. If the mechanist is correct in his assumption, then much remains to be discussed.

Certain other schools of thought have arisen chiefly as escapes from both the fettered philosophy of vitalism and the harshness of mechanism. Such, for example, are *emergent evolution* and its predecessor, *organic determinism*. The contributions of these lie in their emphasis on the concept that a whole is greater than the sum of its parts. Thus, when carbon and oxygen combine to form carbon dioxide, all the properties of carbon have not been united to all the properties of oxygen, but new properties have emerged. The concept is useful, but not new, nor is it an escape from either vitalism or mechanism.

The older position that the distinction between the living and the non-living is sharp and pronounced has been weakened by increased knowledge of highly complex organic substances, such as heavy-weight proteins, enzymes, and viruses. These possess many of the properties of living matter. If in a series of molecules and molecular aggregates grading in complexity from the simplicity of the obviously non-living to the complexity of that which is living, an attempt is made to establish just where life enters, the point cannot be placed. It can, however, be approached. An inorganic molecule is far from being a living particle, whereas a simple organic molecule, like sugar, is a closer approach to life and may represent the first definite step toward it taken ages ago. A highly complex protein molecule is still nearer, for the proteins are not only extremely complex but are of comparatively great size as molecules go, and thus give room for more reactions. Among non-living substances, proteins approach protoplasm most closely in their behavior (page 182).

A typical plant cell, complete in all its parts, may be chosen for the other end of the series. Just below it stands the phloem sieve tube, once a perfect cell, but now devoid of nucleus. Slightly farther down the scale is the mammalian red blood corpuscle, also once a complete cell equipped with all its parts, but now, in the blood stream, it not only lacks a nucleus but is so simplified structurally that there is doubt as to whether it is to be regarded as alive or not. Opinions differ, but the data are sufficient to indicate that the red corpuscle lies somewhere between a typical living cell and a droplet of colloidal matter functioning catalytically.

The bacteria fall very near the dividing line between the living and the non-living because of their simplicity, their great minuteness in size, and their extreme resistance to conditions under which most cells succumb. The midposition between the living and the non-living is best filled by the *viruses.* They possess properties typical of both living and non-living matter. That they are non-living is shown by the fact that they have been isolated, purified, and crystallized, and found to have a molecular weight of 17,000,000. Furthermore, they are definite chemical entities as indicated by their homogeneity, constant composition, optical rotation, isoelectric point, X-ray diffraction pattern, sedimentation constant, absorption spectrum, solubility, pH stability range, and heat-coagulation point. In addition to these properties which are typical of molecules, the virus possesses properties characteristic of organisms for it is able to reproduce, shows specificity of action with respect to host, and ability to induce immunity. Standley concludes that, as the viruses possess certain properties characteristic of organisms as well as the properties of molecules, any attempt to classify them definitely as molecules or as organisms should be one solely of convenience.

With the viruses occupying a midposition between the living and the non-living, a series such as the following may be arranged: inorganic molecules, carbohydrates, simple proteins, non-living enzymes which do not multiply, "enzymes" which multiply in the presence of bacteria, non-pathogenic viruses of the type of bacteriophage, pathogenic viruses, filterable bacteria, visible bacteria, and finally the typical nucleated cell of higher organisms.

From the foregoing series it is evident that the gap between the living and the non-living is not as wide as at first anticipated. The question now arises, is there any gap at all, and if a gap exists, wherein lies the distinction between the matter on the two sides of it? The question cannot be answered, but it is better to organize one's thoughts with a tentative suggestion, a definite philosophy, rather than to leave the entire question open. This can be accomplished by recognizing one of three hypotheses as the most likely one. Unequivocal vitalism will place the responsibility entirely in other hands by recognizing a special vital force or extramundane power. Then there is pure materialism admitting of no distinction between the living and the non-living except complexity, which increases imperceptibly with no abrupt step from that which is living to that which is not. Finally, there is a philosophy "beyond mechanism and vitalism." In accepting the last of the three possibilities, no vitalistic nor superhuman power is resorted to. There is only the recognition that whereas no part of protoplasm is in itself alive or in any way more extraordinary than any other compound of common experience, the whole, as assembled in protoplasm, possesses new properties which, combined, are known as life. Under this assumption there is no supernatural force, and no ingredient peculiar to protoplasm or to life. The constituents of protoplasm may be dealt with by the chemist and the energy relationships determined by the physicist, when they are sufficiently well known. That which is specific to protoplasm is a definite arrangement of its parts, which is peculiar to it and which gives to it a new and distinctive property, or function, namely life.

The arrangement of parts as applied to protoplasm is known as *organization.* The living cell is highly organized, as shown by the great variety of reactions which take place within it without interference one with the other, all within a laboratory of microscopic dimensions.

That a new property or function arises in consequence of a specific organization or structural arrangement of parts may be illustrated by an example. The parts of a clock are all commonplace material, and each piece in its construction is only an ordinary pin, cogwheel, or spring. The aggregate would accomplish nothing if put together haphazardly, but, correctly assembled, the organized whole has the remarkable capacity to keep time.

The moment before the last wheel of the clock is set in place, the unfinished mechanism is but an assemblage of parts devoid of function. With the placing of the last wheel the clock takes on a new quality which is peculiar to it. Just as the clock does not acquire the capacity to keep time unless every part is in its proper place and the whole perfectly organized, so does protoplasm not acquire the property of life unless its parts are assembled into an organized whole.

It may be said, therefore, that the transition from the nonliving to the living is not wholly continuous. The change is accomplished by a discrete and definite step that brings into existence a new world, governed, in part, by a new set of laws, and distinguished from the level below it in the acquisition of a special function, "life."

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